Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Efficacy Trial of a DNA Prime, Adenovirus Type 5 Boost Preventive HIV Vaccine: Supplementary Materials

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1 Clinical Data: Supplementary Materials

1.1 Baseline Participant Characteristics

Table S1 describes the baseline participant characteristics by study arm for the MITT cohort.

1.2 Study Dropout

A total of 175 (7%) of MITT participants terminated the study early. Figure S1 shows the cumulative rate of dropout by study arm. Participants who terminated early are considered to have dropped out immediately following the last completed study visit. Participants not yet diagnosed with HIV-1 infection by the data cut date, completing 24 months of follow-up HIV-1 negative at the Month 24 visit, or diagnosed with HIV-1 infection are censored at the data cut date, 24 months, or the time of HIV-1 diagnosis, respectively. There was a statistically significant higher rate of dropout in placebo recipients

1.3 Behavioral Risk Variables and Risk Score

A behavioral risk score was developed to predict the risk of HIV-1 infection in MITT participants over the course of the trial using a multivariable Cox proportional hazards model. Behavioral risk variables were derived from the behavioral risk questionnaire data from the enrollment visit, which queries about risk behavior over the past 3 months. For 10 participants these data were missing and data from the next visit with a completed questionnaire were used. Quantitative variables were dichotomized at the median. Missing values or "unknown" responses were filled in using single imputation. The number of responses imputed per variable ranged from 4 to 78. Two variables were selected for inclusion in the model based on an all-subsets model selection procedure: an indicator that the number of male sexual partners was greater than 3, and an indicator of unprotected receptive anal sex. The risk score was calculated as a weighted sum of these two risk variables, each weighted by the estimated hazard ratio. The risk score takes a value of 0 if a participant has neither risk factor, 1 if they have both, and an intermediate value (0.46 or 0.54) if they have one or the other. The score itself was highly predictive of HIV-1 infection risk (HR = 6.01 per unit increase, 95% CI: 3.15 to 11.48, $p < 0.001$.

Figures S2-S5 describe the two behavioral risk variables included in the score by study arm and over time since enrollment. There was no evidence of a difference between study arms in frequency of risk behavior at any point in time. For both study arms, the frequency of risk behavior declined following enrollment and the lower frequency was maintained over the course of the study. These patterns were similar for other risk behavior variables (data not shown).

1.4 Safety Analyses

As specified in the Statistical Analysis Plan (SAP), statistical analyses of safety data included all enrolled participants.

1.4.1 Adverse Experiences Within 28 Days of Vaccination

Analyses of adverse experiences (AEs) restricted attention to AEs occurring within 28 days of vaccination. AEs also reported as reactogenicities were not included (double-counted). AEs were analyzed using MedDRA system organ classes and preferred terms. Each participant's reactogenicity/AE was counted

once under the maximum severity or the strongest recorded causal relationship to study product. Table S2 shows the percent of participants in each study arm experiencing local reactogenicity, systemic reactogenicity, and AEs by level of severity. Two-sided Fisher's exact tests were used to compare rates of any local reactogenicity, any systemic reactogenicity, any AE, and any of the above rated severe or greater between study arms.

Rates of each type of AE were also compared between study arms using two-sided Fisher's exact tests. The double false discovery rate (DFDR) method of (Mehrotra and Adewale, 2012) was used to control the false discovery rate at 20%. Table S3 shows the AEs that had unadjusted Fisher's exact p-values less than 0.05, and the single AE that was "flagged" using the DFDR method as having an adjusted p-value less than 0.20. Note that the procedure only produces adjusted p-values for system organ classes that include flagged AEs. As shown in Table S3, one AE was identified as having a different rate among vaccine vs. placebo recipients: injection site pruritus (rate = 1.5% among vaccine and 0.1% among placebo recipients, DFDR-adjusted p = 0.002.)

1.4.2 Participant Deaths

Six participants died during the study. All deaths occurred on the placebo arm and all were judged unrelated to participation in the vaccine trial. The causes of death for these participants were determined to be, respectively: multiple stab wounds, suicide, drug intoxication, acute pancreatitis, myocardial infarction, and site unable to determine.

1.5 Additional Details of the Primary Analysis of Vaccine Efficacy

The SAP specified use of the method of (Lu and Tsiatis, 2008) for the primary analysis of vaccine efficacy, if the best study-arm-pooled model predicting HIV-1 infection (based on the Bayesian information criterion, BIC) had a C-statistic of at least 0.85, and use of the standard Cox model estimator (maximum partial likelihood estimator) and log-rank test if the C-statistic was less than or equal to 0.85. (The C-statistic is interpreted as the probability that a randomly chosen infected participant has a higher risk score than a randomly chosen uninfected participant.) The C-statisic for the best-predicting model, which included the behavioral risk score as the only predictor, was 0.658 for Week 28+ HIV-1 infection and 0.688 for MITT HIV-1 infection. Therefore, the standard approach was used as reported in the main article.

Complementary log-log survival plots were used to check the proportional hazards assumption, for the Week 28+ and MITT cohorts (Figure S6). The tests for violation of proportional hazards yielded p = 0.10 for the Week 28+ cohort and p = 0.26 for the MITT cohort.

1.6 Secondary Analyses of Vaccine Efficacy

1.6.1 Vaccine Efficacy for Preventing MITT HIV-1 Infection

Vaccine efficacy for preventing MITT HIV-1 infection was estimated to be -29.38% (95% CI: -106.28 to 18.85, p = 0.28).

1.6.2 HIV-1 Incidence Over Time

Trends in HIV-1 incidence over the course of the trial were examined. Figure S7 shows estimates of instantaneous hazards of HIV-1 infection over time for the vaccine and placebo groups, for the Week 28+ and MITT cohorts (Gilbert et al., 2002). In both cohorts, HIV-1 incidence was relatively constant over time; the wide confidence intervals do not provide support for any significant time trends in incidence.

1.6.3 Vaccine Efficacy by Baseline Subject Characteristics

A set of secondary analyses was performed to address possible modification of the vaccine effect on HIV-1 acquisition by baseline subject characteristics. Table S4 shows the estimates VE for preventing Week 28+ HIV-1 infection using Cox regression partial likelihood estimation by baseline variables. Table S5 shows parallel results for the MITT cohort. There was some suggestion that the vaccine increased the HIV-1 infection rate among subjects with high BMI, but this unplanned/post-hoc subgroup analysis must be interpreted with caution, especially given that the p-value for the test of interaction was only borderline significant ($p = 0.14$ for the Week 28+ cohort, $p = 0.05$ for the MITT cohort), even without adjustment for the multiple subgroup analyses.

1.6.4 Vaccine Efficacy in the Per-Protocol Cohort

The methods of (Gilbert et al., 2013) were used to estimate cumulative vaccine efficacy in the always per-protocol subcohort over time, where cumulative VE by time t since enrollment is one minus the ratio of the probabilities of being diagnosed with HIV-1 infection by time t (vaccine / placebo). Figure S8 shows the estimates of vaccine efficacy over time in the always per-protocol, Week 28+, and MITT cohorts, with pointwise 95% confidence intervals. The per-protocol VE estimates are highly consistent with the Week 28+ estimates; and none of the VE estimates, in any cohort or at any point in time, significantly differed from zero.

1.6.5 Sensitivity of Vaccine Efficacy Results to Assumptions Regarding Participant Dropout

Two sets of sensitivity analyses were performed to asses what the vaccine:placebo HR for Week 28+ HIV-1 infection would be, and what the p-value for testing for a HR greater than one would be, if participants who dropped out had not done so. The analyses made different assumptions about what would have been the infection rate among dropouts. Specifically, we considered the following 15 scenarios with annual HIV-1 infection rates for dropouts in the vaccine arm and placebo arm:

- Scenarios 1-5: Same annual infection rates for vaccine and placebo dropouts: (0, 0), (0.025, 0.025), (0.05, 0.05), (0.075, 0.075), (0.1, 0.1)
- Scenarios 6-10: No infections among vaccine dropouts: (0, 0.015), (0, 0.025), (0, 0.05), (0, 0.075), (0, 0.1)
- Scenarios 11-15: No infections among placebo dropouts: (0.015, 0), (0.025, 0), (0.05, 0), (0.075, 0), (0.1, 0)

The annual infection rates we explored were chosen based on the observed annual incidence of Week 28+ HIV-1 infection in the study (2.3% among placebo and 2.8% among vaccine recipients).

Additional sensitivity analyses that incorporate information from the baseline behavioral risk score were conducted. This set of sensitivity analyses considered the possibility that the HIV-1 infection rate among participants who dropped out of the study varied according to their risk score. Specifically, for each dropout, we simulated their unobserved infection time during the subsequent follow-up period from an exponential distribution with a mean equal to the infection rate specified in the above scenarios, multiplied by the HR of infection contrasting the participant's risk score to the low risk category. We only considered scenarios where vaccine and placebo dropouts had the same infection rates (Scenarios 1-5).

A total of 100 placebo recipients and 75 vaccine recipients dropped out of the study before completing the 24 months of follow-up. For participants who were observed to drop out, we simulated their unobserved

infection time during the subsequent follow-up period from an exponential distribution with a specified mean annual infection rate. Those dropouts who had a simulated unobserved infection time exceeding 24 months were censored at 24 months. For each scenario, we analyzed 1000 trials and we report the distribution of one-sided p-values for testing H_0: HR$\leq$1. Figure S9 shows the results for scenarios 1-15 ignoring the behavioral risk score information, and for scenarios 1-5 incorporating the behavioral risk score information. The results of the scenarios considered suggest that the vaccine was not enhancing HIV-1 infection risk. Enhancement would only be supported under the extreme assumption that there were no HIV-1 infections among placebo dropouts and infections among vaccine dropouts at a rate of at least 3 times the pooled HIV-1 infection rate. Conclusions regarding all MITT infections were similar (data not shown).

1.7 Additional Details on the Primary Analysis of Setpoint Viral Load

HIV-infected participants were evaluable for the analysis of setpoint viral load (VL) if they had at least one post-infection visit with VL and CD4+ T cell count measured. Out of 48 Week 28+ infected participants, 0 vaccine and 1 placebo recipients were not evaluable. Out of 72 MITT infected participants, 1 vaccine and 2 placebo recipients were not evaluable.

Of the 47 evaluable Week 28+ infected subjects, 39 had observed setpoint VL endpoints (22 vaccine and 17 placebo recipients). Of the 69 evaluable MITT infected subjects, 57 had observed setpoint VL endpoints (34 vaccine and 23 placebo recipients). Table S6 shows the reasons for missing setpoint VL data among evaluable Week 28+ and MITT infected participants by study arm.

The setpoint VL endpoint is an average of all available VL measurements from Weeks 10, 12, 14, 16, and 20 PD (5 visits), resticting to samples drawn prior to initiation of ART. Among Week 28+ infected participants with observed setpoint VL endpoints, the number of VL measurements contributing to the setpoint VL average ranged from 1 to 5 (median = 4) among vaccine recipients, and 1 to 5 (median = 5) among placebo recipients. Among MITT infected participants with observed setpoint VL endpoints, the number of VL measurements contributing to the setpoint VL average ranged from 1 to 5 (median = 4) among vaccine recipients, and 1 to 5 (median = 5) among placebo recipients.

The SAP specified use of the robust likelihood-based method of (Little and An, 2004) for analysis of setpoint VL. This method is designed to minimize potential bias due to missing VL endpoint data by accounting for observed variables that predict VL endpoint missingness. (Gilbert and Jin, 2010) extended this method to allow for the possibility of post-randomization selection bias. As specified in the SAP, for the primary VL analysis we assumed no selection bias ($\beta_0 = \beta_1 = 0$) and no individual-level benefit from vaccination ($\phi = p_0/p_1$), consistent with the estimate of VE.

Implementation of the method involves modeling the probability of having a missing setpoint VL measurement. We factored the probability of having an observed VL endpoint as: the probability of not dropping out prior to Week 10 post-infection diagnosis (PD), times the probability of not initiating antiretroviral therapy (ART) prior to Week 10 PD conditional on not dropping out prior to Week 10, times the probability of not missing all visits Week 10-20 PD. Each of these probabilities was estimated empirically by study arm; there were insufficient data to permit modeling of additional covariate effects. The method also involves imputing the missing setpoint VL values. The imputation model was determined by an all-subsets linear regression model selection procedure as specified in the SAP. The only variable found to predict setpoint VL was average pre-week 10 viral load for both Week 28+ infections and MITT infections.

1.8 Secondary Analyses of Viral Load

1.8.1 Vaccine Effect on Setpoint Viral Load in MITT Infected Participants

The estimated vaccine effect on setpoint VL among MITT infected participants was 0.03 (95% CI: -0.42 to 0.48, p = 0.90).

1.8.2 Vaccine Effect on Setpoint Viral Load by Baseline Subject Characteristics

A set of secondary analyses was performed to address possible modification of the vaccine effect on setpoint VL by baseline covariates. Table S7 shows the results of the setpoint VL analysis for Week 28+ HIV-1 infections by baseline variables. Table S8 shows the parallel results for all MITT infections. There was some suggestion that the vaccine lowered setpoint VL among subjects with high BMI, particularly among Week 28+ infections, but again this unplanned/post-hoc subgroup analysis must be interpreted with caution, especially given the borderline significance of the tests of interaction (p = 0.03 for Week 28+ infections, p = 0.15 for MITT infections), even unadjusted for the multiple subgroup analyses.

1.9 Longitudinal Viral Load and CD4+ T Cell Count

Viral load was measured at visits at Weeks 0, 2, 4 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, 40, 44, 48, 56, 64, and 72 post-infection diagnosis (PD). CD4+ T cell count was measured at visits at Weeks 2, 4, 12, 16, 20, 24, 32, 40, 48, 56, 64, and 72 PD. Figures S10 and S11 show longitudinal pre-ART VL and CD4+ T cell count trajectories over Weeks 0-20 PD in the Week 28+ infected cohort. Figures S12 and S13 show parallel results for the MITT infected cohort. There is no evidence of a meaningful difference in mean pre-ART VL or CD4+ T cell count between study arms at any point in time, either among Week 28+ or MITT infections.

2 Immunological Data: Supplementary Materials

2.1 Additional Details on Immunological Data Sampling Plan

A total of 40 vaccine and 10 placebo recipients were selected at random from the 403 participants (199 placebo recipients and 204 vaccine recipients) who had reached the Month 24 visit HIV-1 uninfected, and who had a negative HIV-1 test result at the Month 24 visit, at the time of sampling (mid October, 2012). Eligible participants were further required to have:

1. Received all 4 study vaccinations with correct product administration,
2. Specimens taken at each of the 4 main immunogenicity time points (Months 0, 7, 12, 24), and
3. A sufficient quantity of each of the three specimen types (serum, plasma, PBMCs) available at each of the 4 immunogenicity time points.

For criterion (3), a participant missing an immunogenicity visit implied insufficient quantity. For attended visits, the minimum specimen quantity was set to approximately the third percentile of the observed volume/cell-count distribution, i.e., 97% of subjects had volumes/cell-counts greater than this amount. The distribution of volumes/cell counts was based on specimens for all participants, not just those meeting the criteria above.

PBMC specimens for one of the selected vaccine recipients were destroyed due to improper storage at the central repository, leaving samples from 49 participants (39 vaccine and 10 placebo recipients) for the intracellular cytokine staining assay analysis. The binding antibody and neutralizing antibody analyses, which use serum and plasma specimens, were based on the complete set of 50 participants.

2.2 Additional Details on Laboratory Methods

2.2.1 Intracellular Cytokine Staining Assay

To evaluate vaccine-induced T cell responses at visit 7 (Day 196; four weeks after the rAd5 vaccination), a validated intracellular cytokine staining assay (ICS) was performed as previously described (Horton et al., 2007) with modifications. Previously cryopreserved peripheral blood mononuclear cells (PBMC) (10^6) were stimulated for 6 hours with pools of 15 amino acid peptides matched to the sequence of the vaccine inserts (VRC GagB, PolB, NefB, EnvA, EnvB and EnvC). Stimulation with phytohemagglutinin (PHA, 1 μg/ml) and 0.5% dimethyl sulfoxide (DMSO) diluted in R10 media (RPMI 1640 [Gilbco-Bethesda Research Laboratories] containing 10% fetal calf serum [Gemini Bioproducts]) served as positive and negative controls, respectively.

The following antibodies were used for fluorescent staining: CD8-PerCP-Cy5.5, IL-2-PE, IFN-γ-V450, TNF-α-FITC, CD154-PE-Cy5, IL-4-APC, GranzymeB-Alexa700, CD107a-PE-Cy7 (all Becton Dickinson Biosciences (BD), San Jose, CA); CD4-APC-Alexa750 and CD3-ECD (Beckman Coulter, Miami, FL); CD14-Qdot655 (Invitrogen); and Fixable Aqua Dead Cell Stain (Invitrogen; Eugene, OR). The BD Cytofix/Cytoperm kit protocol was used for cell fixation and permeabilization. Samples were acquired on a LSRII flow cytometer (BD) and analyzed using FlowJo software (Treestar, Ashland, OR). Assays were run under Good Clinical Laboratory Practice (GCLP) compliant conditions.

2.2.2 Binding Antibody Multiplex Assay

Serum HIV-1 specific antibodies (IgG and IgA) were measured by a validated HIV-1 binding antibody multiplex assay (BAMA) as previously described (Tomaras et al., 2008; Haynes et al., 2012) at visit 2 (Day 0) and visit 7. The following HIV-1 Env antigens were tested (the abbreviated names reported in Figure S15 are given in parentheses):

Con S gp140 CFI (Con S)
Con 6 gp120/B (Con 6 B)
gp70_B.CaseA2_V1_V2 (gp70 (case A2))
gp70-V1V2 (A) (gp70 V1V2 (VRC A))
B.HXB/Bal_140CFI_avi/293F (VRC B)
A.92RW020_140CFI_Avi/293F (VRC A)
C.97ZA012_140CFI_Avi/293F (VRC C).

These proteins were provided by Drs. Liao and Haynes, Duke Human Vaccine Institute, supported by Bill & Melinda Gates Foundation; gp70V1/V2 caseA2 sequences provided by Dr. A. Pinter, NJMS-Rutgers; and clade B gp41 (Immunodiagnostics). All assays were run under GCLP compliant conditions, including tracking of positive controls by Levy-Jennings charts using 21CFR Part 11 compliant software. Positive controls included a purified polyclonal IgG from HIV subjects (HIVIG) titration, and CH58 monoclonal antibody (Liao et al., 2013) IgG titration. The negative controls were NHS (HIV-1 sero-negative human sera) and blank beads. For IgG, serum titrations from 1:50 to 1:156250 (six 5-fold dilutions 50, 250,

1250,6250, 31250, and 156250) were performed to provide a sample within the linear range of the standard curve for calculating Area Under the titration Curve (AUC), which measures the magnitude of binding antibodies. The statistical methods section below describes the algorithm for computing the AUC.

2.2.3 Neutralizing Antibody Assay

Neutralizing antibody responses at visit 7 were measured in a formally optimized and validated assay in compliance with GCLP, including successful participation in a validated proficiency testing program designed specifically for this assay (Todd et al., 2012). In this assay, virus neutralization is quantified as a function of reductions in luciferase (Luc) reporter gene expression after a single round of infection in TZM-bl cells (Montefiori, 2012; Li et al., 2005). TZM-bl cells (also called JC57BL-13) were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, as contributed by John Kappes and Xiaoyun Wu. This is a HeLa cell clone that was engineered to express CD4 and CCR5 (Platt et al., 1998) and to contain integrated reporter genes for firefly luciferase and E. coli beta-galactosidase under control of an HIV-1 LTR (Wei et al., 2002). Briefly, a pre-titrated dose of virus was incubated with serial 3-fold dilutions of test sample in duplicate in a total volume of 150 μl for 1 hour at 37°C in 96-well flat-bottom culture plates. Freshly trypsinized cells (10,000 cells in 100 μl of growth medium containing 75 μg/ml DEAE dextran) were added to each well. One set of control wells received cells + virus (virus control) and another set received cells only (background control). After 48 hours of incubation, 100 μl of cells was transferred to a 96-well black solid plate (Costar) for measurements of luminescence using the Britelite Luminescence Reporter Gene Assay System (PerkinElmer Life Sciences). Assay stocks of molecularly cloned Env-pseudotyped viruses were prepared by transfection in 293T/17 cells (American Type Culture Collection) and titrated in TZM-bl cells as described (Montefiori, 2012; Li et al., 2005). Additional information on the assay and all supporting protocols may be found at: http://www.hiv.lanl.gov/content/nab-reference-strains/html/home.htm.

2.3 Additional Details on Statistical Methods

Data for all assays were excluded from analysis if the blood draw date was not within the allowable visit window as determined by the protocol.

2.3.1 Intracellular Cytokine Staining Assay Data Analysis

Several criteria were used to determine if data from the assay were acceptable and could be analyzed. On the second day after sample thawing, the viability must have been 66% or greater. If not, the sample was retested. If upon retesting the viability remained below this threshold, no data were included for this sample. For the negative control acceptance criteria, if the average cytokine response for the negative control wells was above 0.1% for either the CD4+ or CD8+ T cells, then the sample was retested. If the retested results were above 0.1% then the data were excluded from analysis; otherwise the retest data were used. In addition, the total number of CD4+ and CD8+ T cells was required to exceed certain thresholds. If the number of CD4+ or CD8+ T cells was less than 5,000 for any of the HIV-1 peptide pools or one of the negative control replicates for a particular sample, data for that stimulation was filtered. If both negative control replicates were less than 5,000 cells, the sample was retested. If upon retesting, one negative control replicate was less than 5,000, the negative control replicate with more than 5,000 cells was used. If both negative control replicates from the retest for a T-cell subset were less than 5,000, then data for the T-cell subset were not included in the analysis.

Positivity for a peptide pool within a T-cell subset, determined by the percent of cells expressing IL-2 and/or IFN-γ, was determined using previously described methods (Horton et al., 2007). If at least one peptide pool for a specific HIV-1 protein was positive, then the overall response to the protein was considered positive. If any peptide pool was positive for a T-cell subset, then the overall response for that T-cell subset was considered positive. Confidence intervals for response rates were calculated using the score test method (Agresti and Coull, 1998).

The magnitude of the response to a peptide pool within a T-cell subset was defined as the net percentage of cells expressing IL-2 and/or IFN-γ, peptide-stimulated minus unstimulated. Because of peptide overlaps between peptide pools for the Env protein, the magnitude for Env was calculated as the maximum among the Env peptide pools. The Pol peptides did not overlap and magnitude was calculated as the sum of the Pol peptide pools. The overall magnitude was calculated as the sum of the individual HIV-1 protein magnitudes.

Response rates were compared between the vaccine and placebo groups using Fisher's exact tests, and distributions of magnitudes of responses among positive responders were compared using Wilcoxon rank sum/Mann-Whitney tests. The p-values reported from these tests were not adjusted for multiple testing.

2.3.2 Binding Antibody Multiplex Assay Data Analysis

The readout of the assay is background-subtracted mean fluorescent intensity (MFI), where background refers to a plate level control (i.e., a blank well run on each plate or MulVgp70_His6 for the 3 MulVgp70 scaffolded antigens).

The following criterion was used to determine if the data for a sample was included in the analysis. If the blank bead negative control readout exceeded 5,000 MFI, the sample was retested. If the retest value exceeded 5,000 MFI, the sample was excluded from analysis due to high background.

Samples from visit 7 were declared to be positive if they met three conditions: (1) the MFI minus blank values were $\geq$ an antigen-specific cutoff at the 1:50 dilution level based on the average + 3 standard deviations of at least 60 seronegative plasma samples; (2) the MFI minus background/blank values were greater than 3 times the baseline (visit 2, pre-immunization) MFI minus background/blank values; and (3) the MFI values were greater than 3 times the baseline MFI values.

The magnitude of the response was measured by the background-subtracted MFI at the 1:50 dilution level, and is referred to as the "net response".

Based on the titration experiments that measured background-subtracted MFI at each of six 5-fold dilutions ranging from 1:50 to 1:156250, the AUC (Area Under the titration Curve) value was calculated using the trapezoidal rule, where the AUC is a sum of the area of trapezoids calculated between any non-excluded data points in the analyte/titration.

Statistical methods for performing inference about the response rates and magnitudes of response were the same as reported above for the ICS assay.

2.3.3 Neutralizing Antibody Multiplex Assay Data Analysis

The magnitude of the response to a virus, called the "neutralizing antibody titer" or inhibitory dose 50% (ID50), was defined as the serum dilution that reduced relative luminescence units (RLUs) by 50% relative to the RLUs in virus control wells (cells + virus only), after subtraction of background RLU (cells only).

A positive response to a virus was defined to be a neutralizing antibody titer of at least 10.

Statistical methods for performing inference about the response rates and magnitudes of response were the same as reported above for the ICS assay.

2.4 Additional Immunological Results

2.4.1 Intracellular Cytokine Staining Assay Results

Figure S14 shows the distribution of HIV-1-specific CD4+ and CD8+ T-cell responses at visit 7 to vaccine-matched HIV-1 peptide pools for vaccine and placebo recipients.

2.4.2 Binding Antibody Multiplex Assay Results

Figure S15 shows the distribution of binding antibody responses against Env antigens at visit 7 for vaccine and placebo recipients. Figure S16 shows the distribution of IgG binding antibody AUCs for vaccine recipients.

2.4.3 Neutralizing Antibody Assay Results

Figure S17 shows the distribution of neutralizing antibody titers against Tier 1 isolates at visit 7 for the vaccine and placebo arms.

3 Updated Analysis of the Vaccine Effect on HIV-1 Infection

After the HVTN 505 trial was unblinded on April 23, 2013, follow-up continued using the same protocol-specified procedures in place prior to unblinding. A new version of the protocol (Version 5.0) was developed, which included a plan for approximately 6-monthly analyses, to assess the vaccine effect on HIV-1 infection based on updated data, pooling over data collected before and after April 23, 2013. These analyses are reviewed by the HVTN 505 Oversight Committee. The first planned analysis took place on September 3, 2013. The statistical report for this analysis included data entered into the data base through August 23, 2013. The rate of retention of study participants remained high, with annual dropout incidence over all follow-up of 0.051 (95% CI 0.042 to 0.062) in the vaccine arm and 0.070 (95% CI 0.059 to 0.084) in the placebo arm.

Figures S18 and S19 show the Kaplan-Meier curves of the cumulative incidence of HIV-1 infection through the Month 24 visit by study arm for the Week 28+ and MITT cohorts, respectively, computed identically to those in Figure 2 of the main article based on the updated data base. For each cohort analysis the rates of HIV-1 infection were similar in the vaccine and placebo groups, with log-rank test p-values 0.76 and 0.56 for the Week 28+ and MITT analyses, respectively.

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5 HVTN 505 Study Team

Table S9 contains the HVTN 505 study team.

Figures

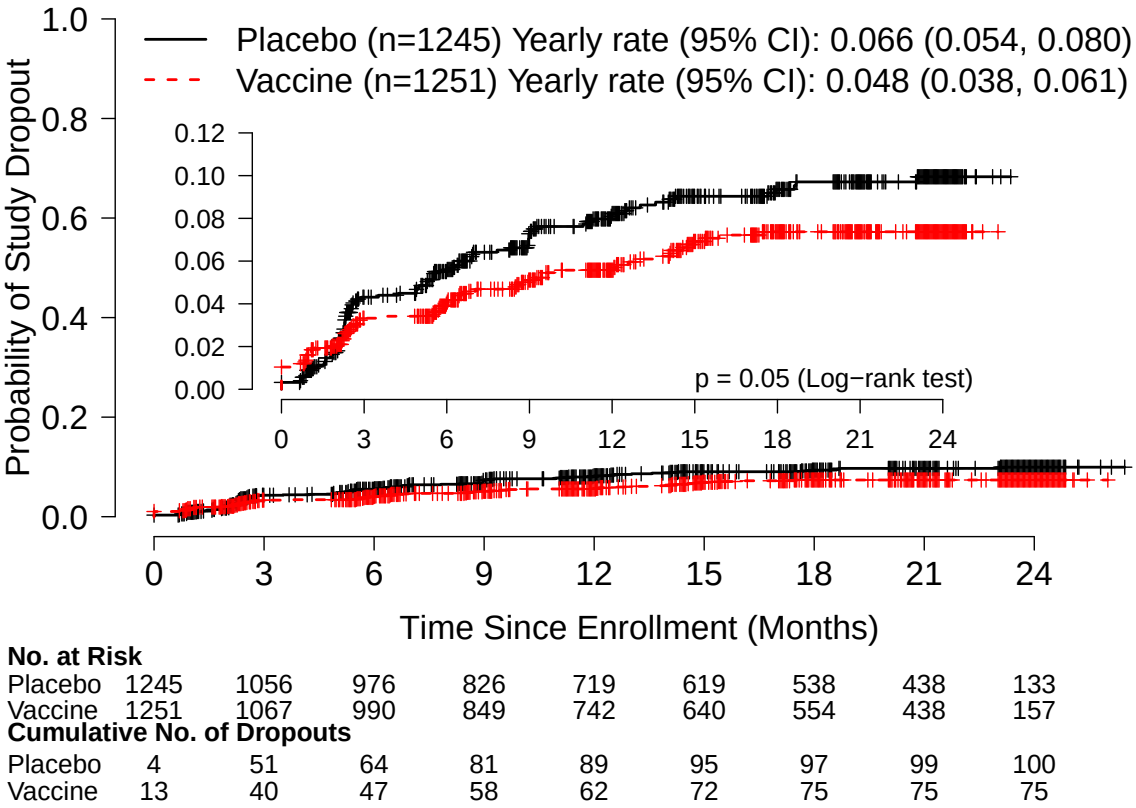


Figure S1: Cumulative incidence of dropout by study arm among participants in the MITT cohort.

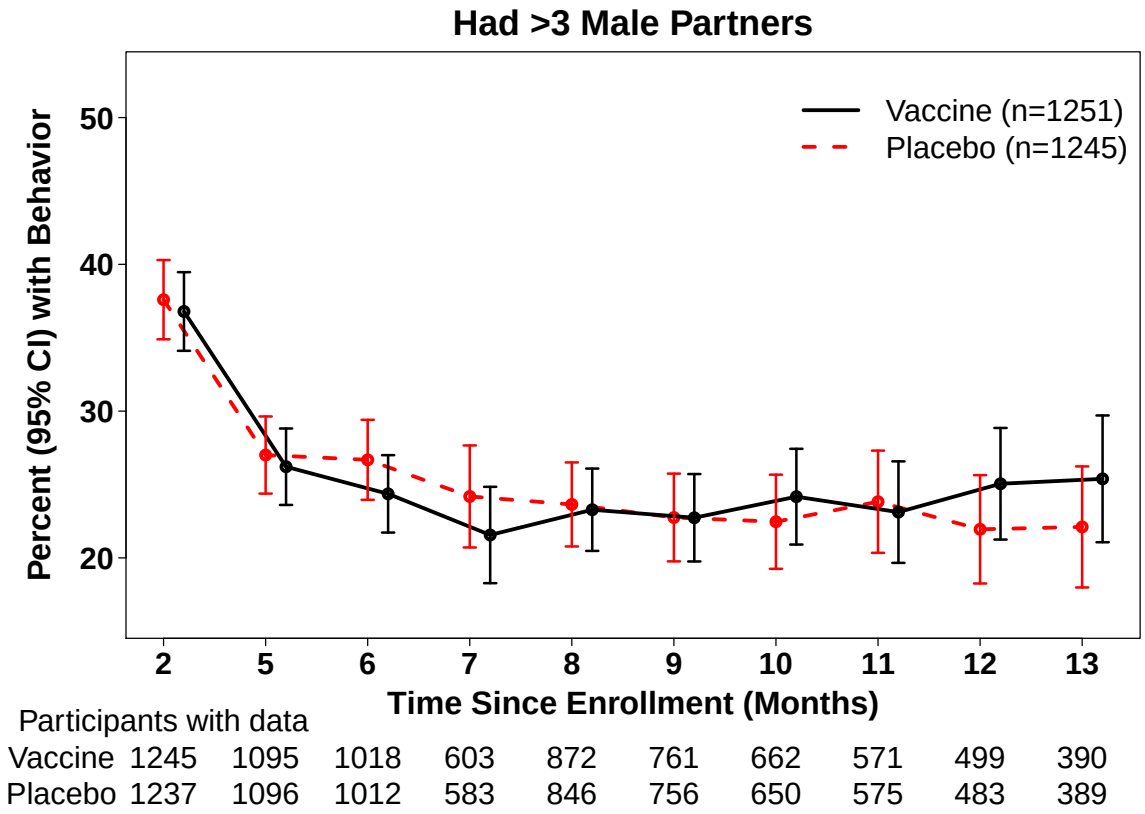


Figure S2: Percent of MITT participants reporting more than 3 male sexual partners by study arm and time since enrollment. Vertical lines represent pointwise 95% confidence intervals.

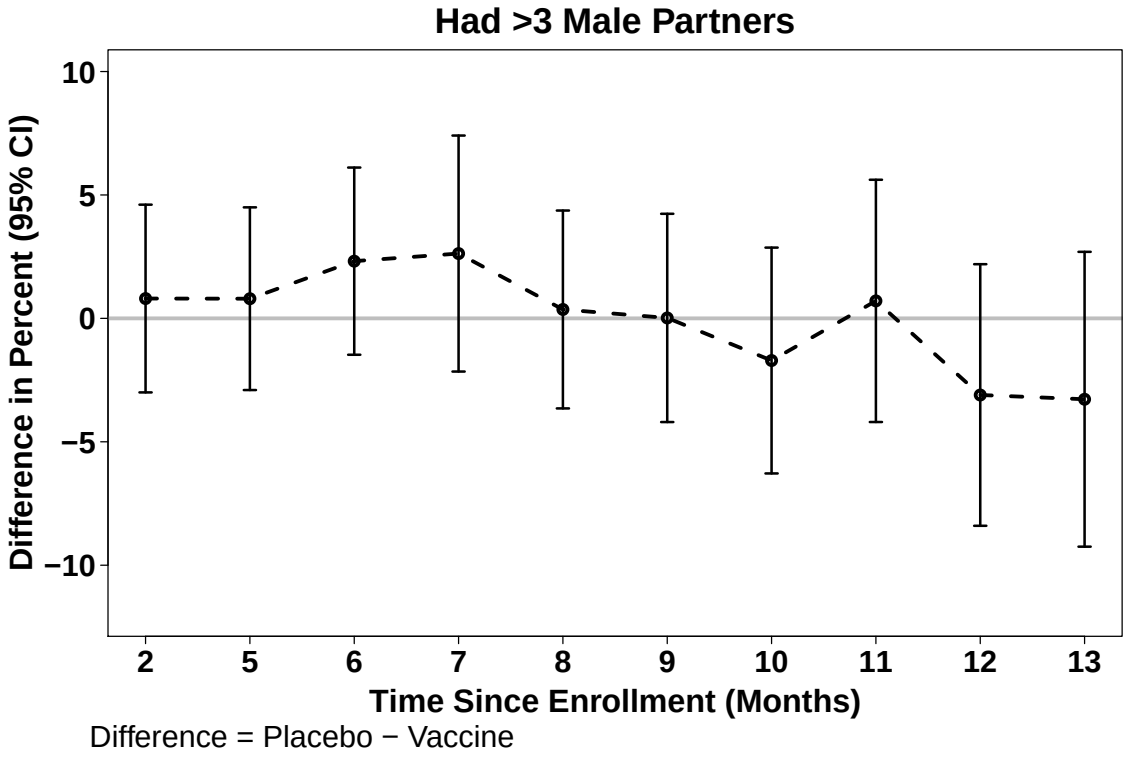


Figure S3: Difference in percent of MITT participants reporting more than 3 male sexual partners (Placebo - Vaccine) by time since enrollment. Vertical lines represent pointwise 95% confidence intervals.

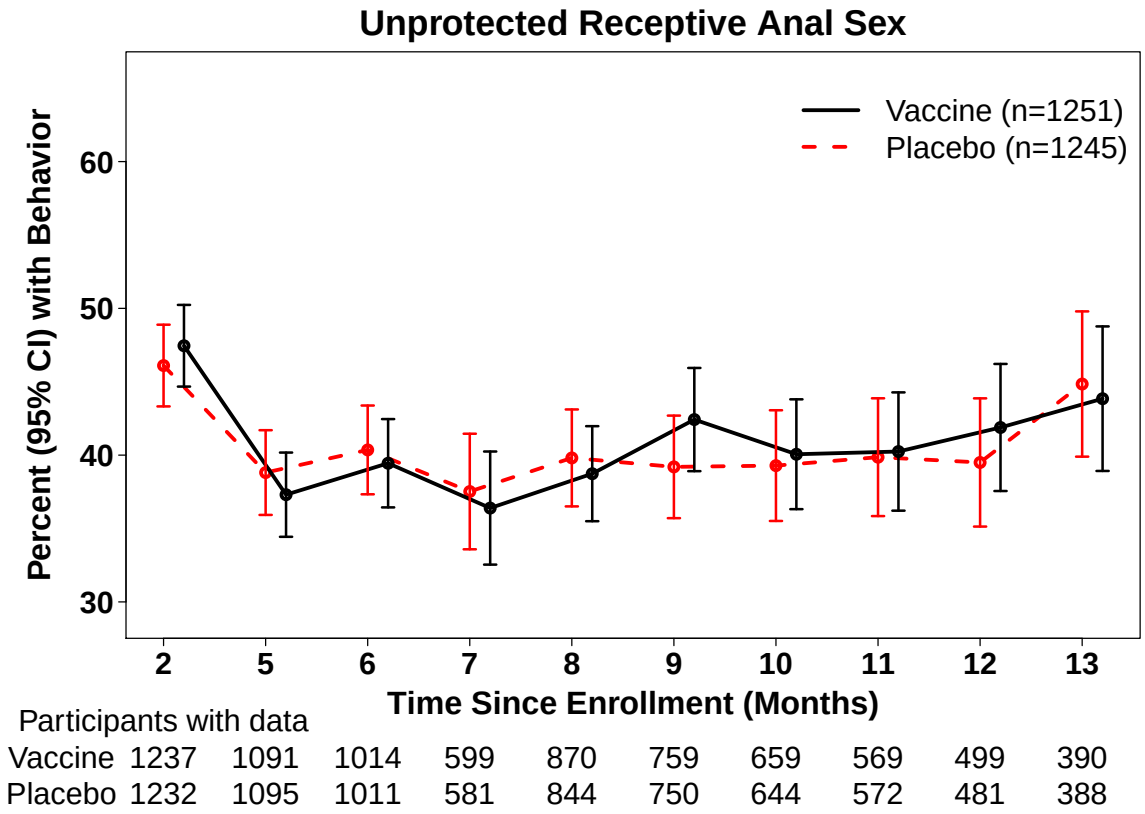


Figure S4: Percent of MITT participants reporting having unprotected receptive anal sex by study arm and time since enrollment. Vertical lines represent pointwise 95% confidence intervals.

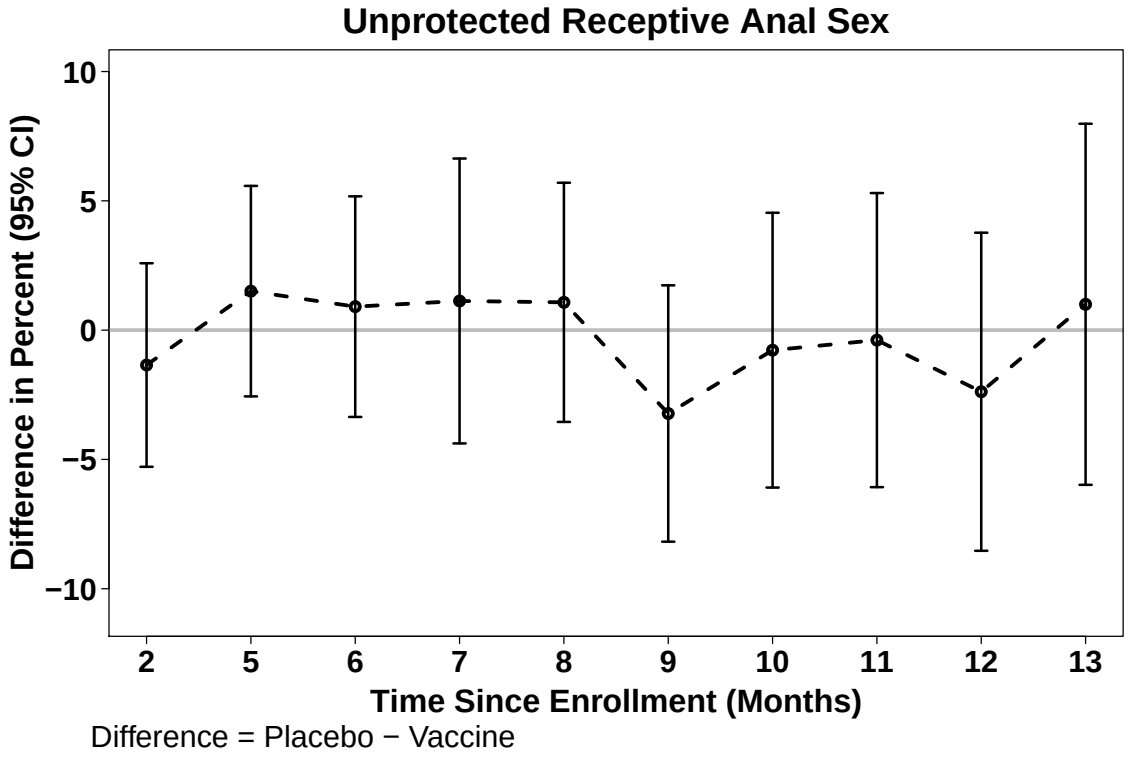


Figure S5: Difference in percent of MITT participants reporting having unprotected receptive anal sex (Placebo - Vaccine) by time since enrollment. Vertical lines represent pointwise 95% confidence intervals.

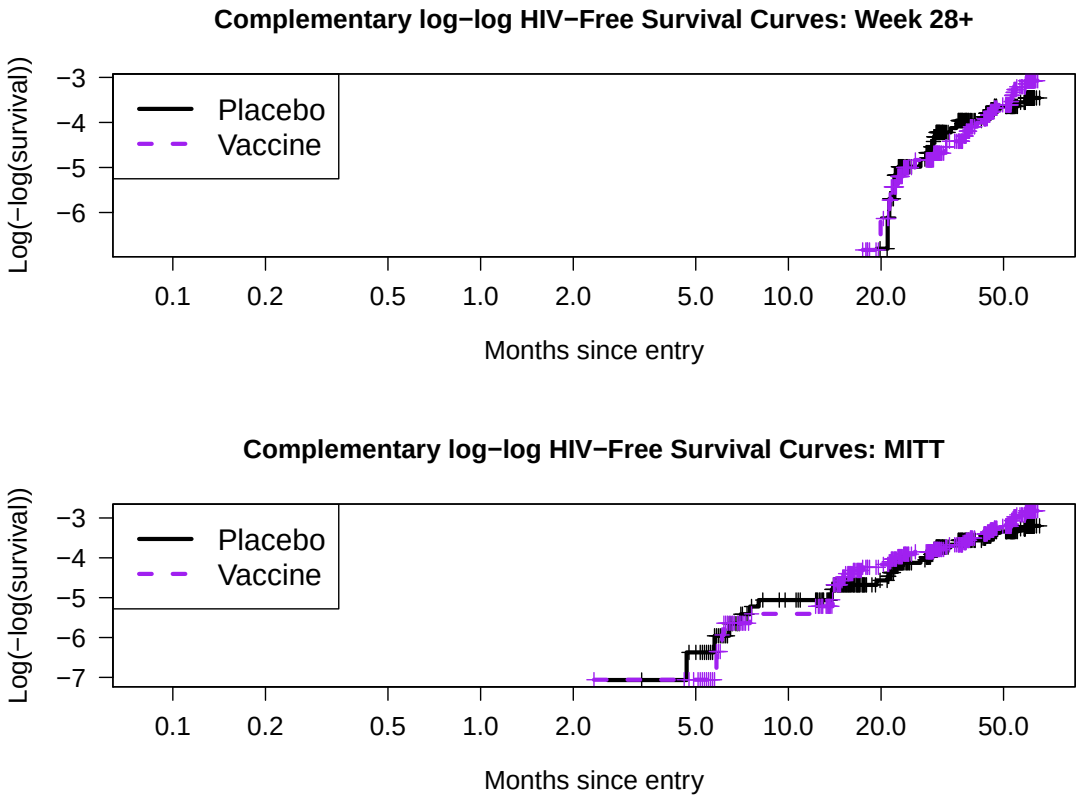


Figure S6: Complementary log-log survival plots to diagnose the proportional hazards assumption for the Week 28+ and MITT cohorts.

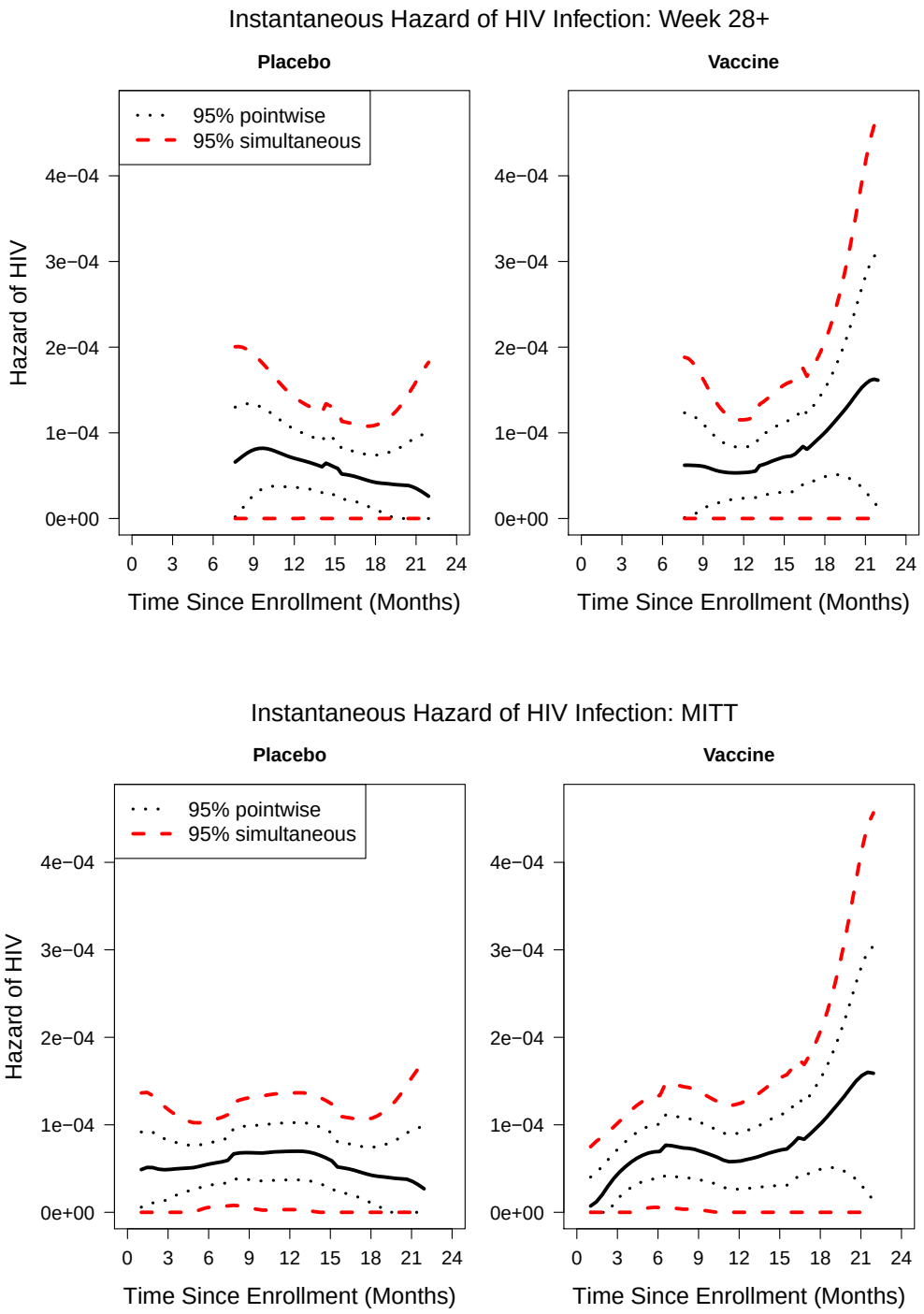


Figure S7: Instantaneous hazard of HIV-1 infection in the vaccine and placebo groups, for the Week 28+ cohort (top) and MITT cohort (bottom).

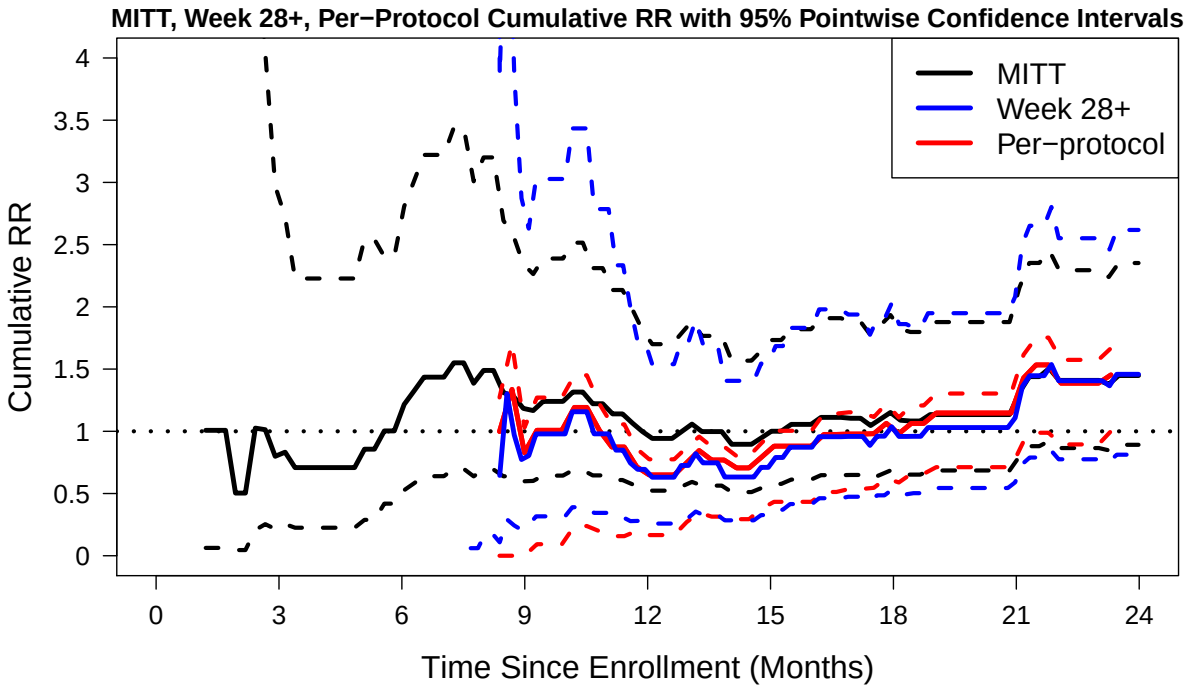


Figure S8: Comparison of Week 28+, MITT, and per-protocol estimates of vaccine efficacy under assumption set D in Gilbert et al. (2013).

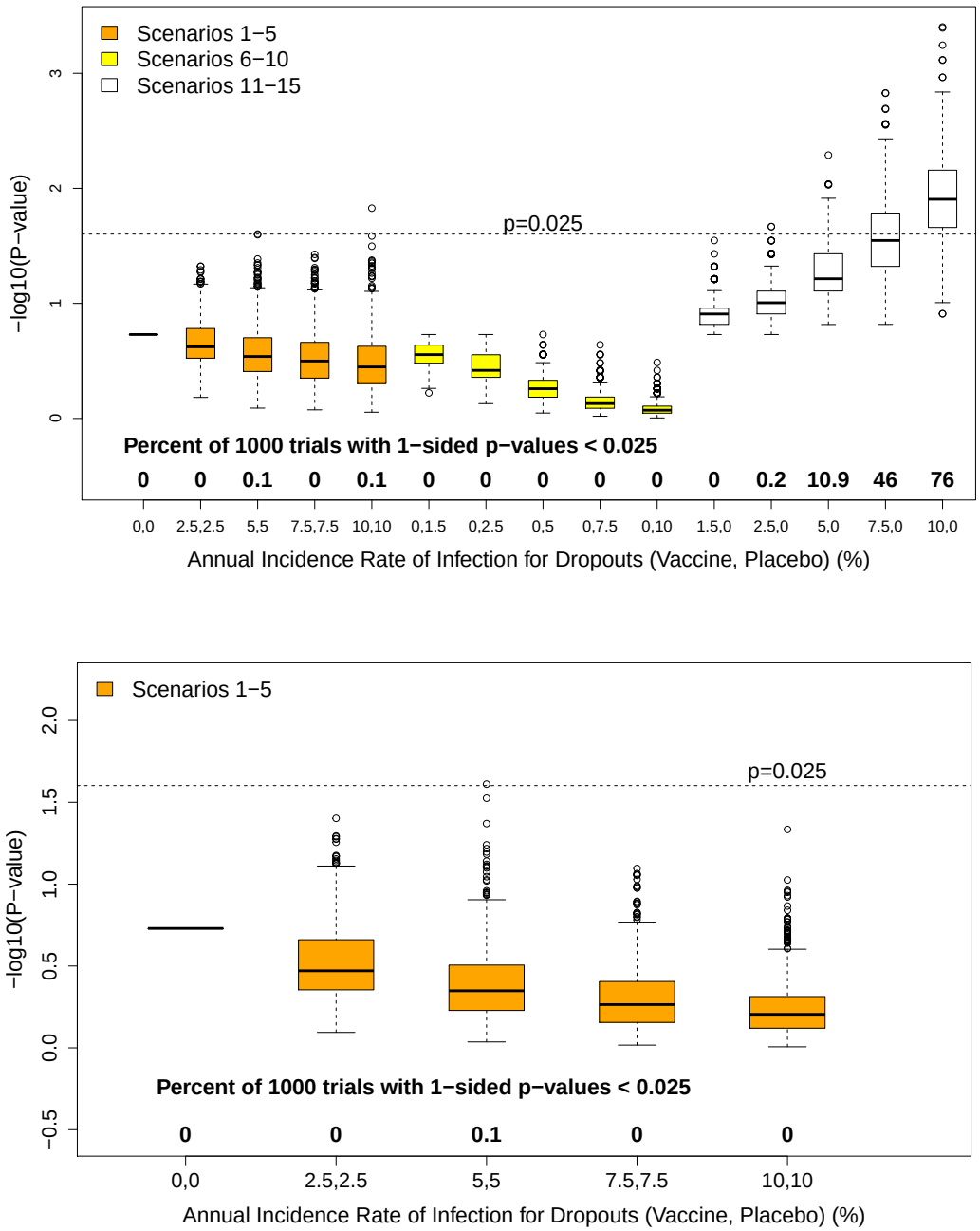


Figure S9: Sensitivity analyses: Boxplots of one-sided p-values testing for an elevated rate of Week 28+ infection in the vaccine vs. placebo arm (i.e., testing H_0 : HR ≤ 1 vs. H_1 : HR > 1), ignoring the behavioral risk score information (top) and incorporating the behavioral risk score information (bottom).

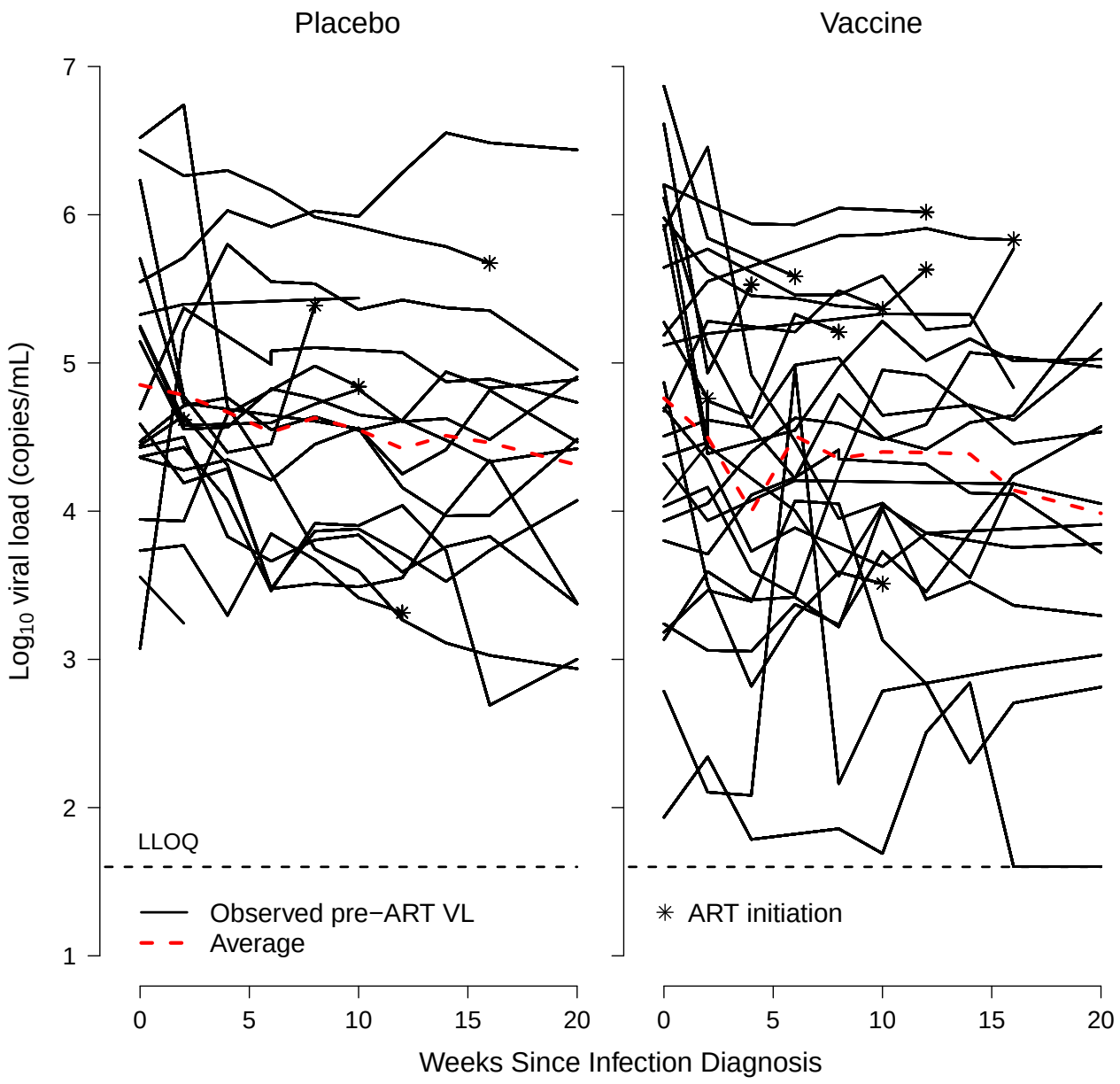


Figure S10: Longitudinal pre-ART $\log_{10}$ viral load trajectories over weeks 0-20 post-infection diagnosis for individual Week 28+ infected participants, by study arm. Red lines connect averages at each post-infection diagnosis visit with at least 3 observed values. Stars indicate participants whose viral load trajectories were censored by ART initiation. The lower limit of quantification (LLOQ) of the assay is 40 copies/mL.

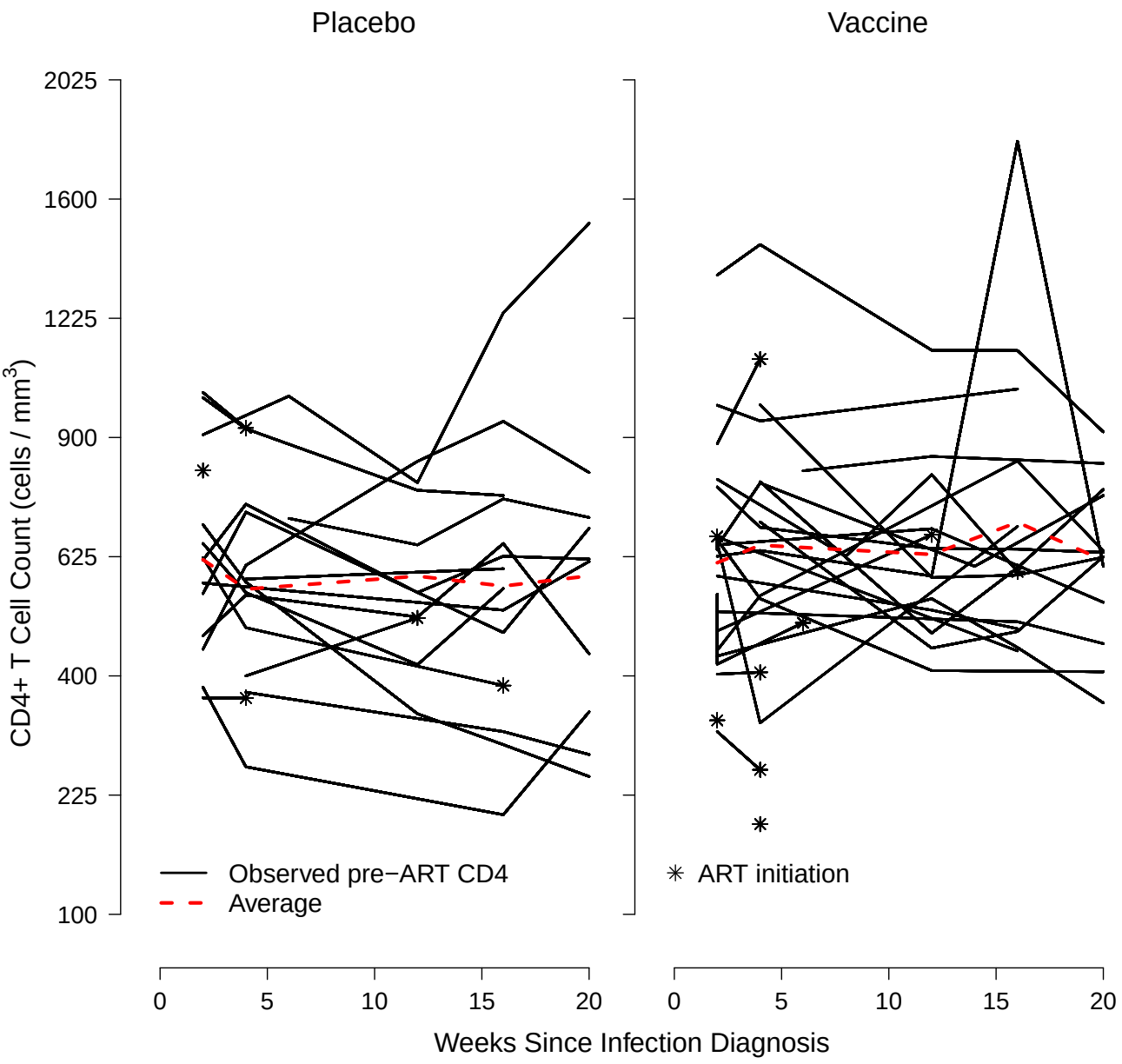


Figure S11: Longitudinal pre-ART CD4+ T cell count trajectories over weeks 2-20 post-infection diagnosis for individual Week 28+ infected participants, by study arm. Red lines connect averages at each post-infection diagnosis visit with at least 3 observed values. Stars indicate participants whose viral load trajectories were censored by ART initiation.

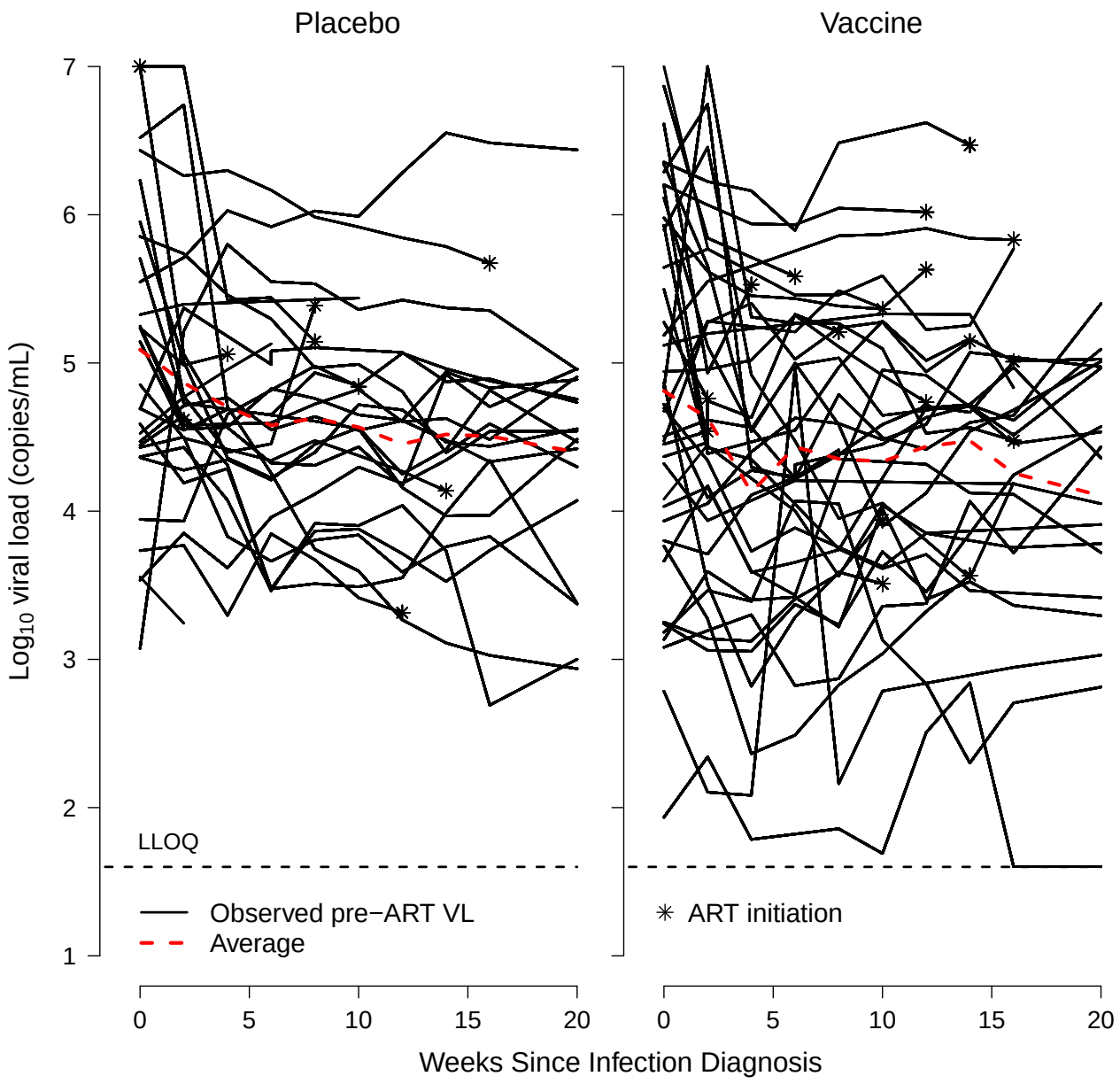


Figure S12: Longitudinal pre-ART $\log_{10}$ viral load trajectories over weeks 0-20 post-infection diagnosis for individual MITT infected participants, by study arm. Red lines connect averages at each post-infection diagnosis visit with at least 3 observed values. Stars indicate participants whose viral load trajectories were censored by ART initiation. The lower limit of quantification (LLOQ) of the assay is 40 copies/mL.

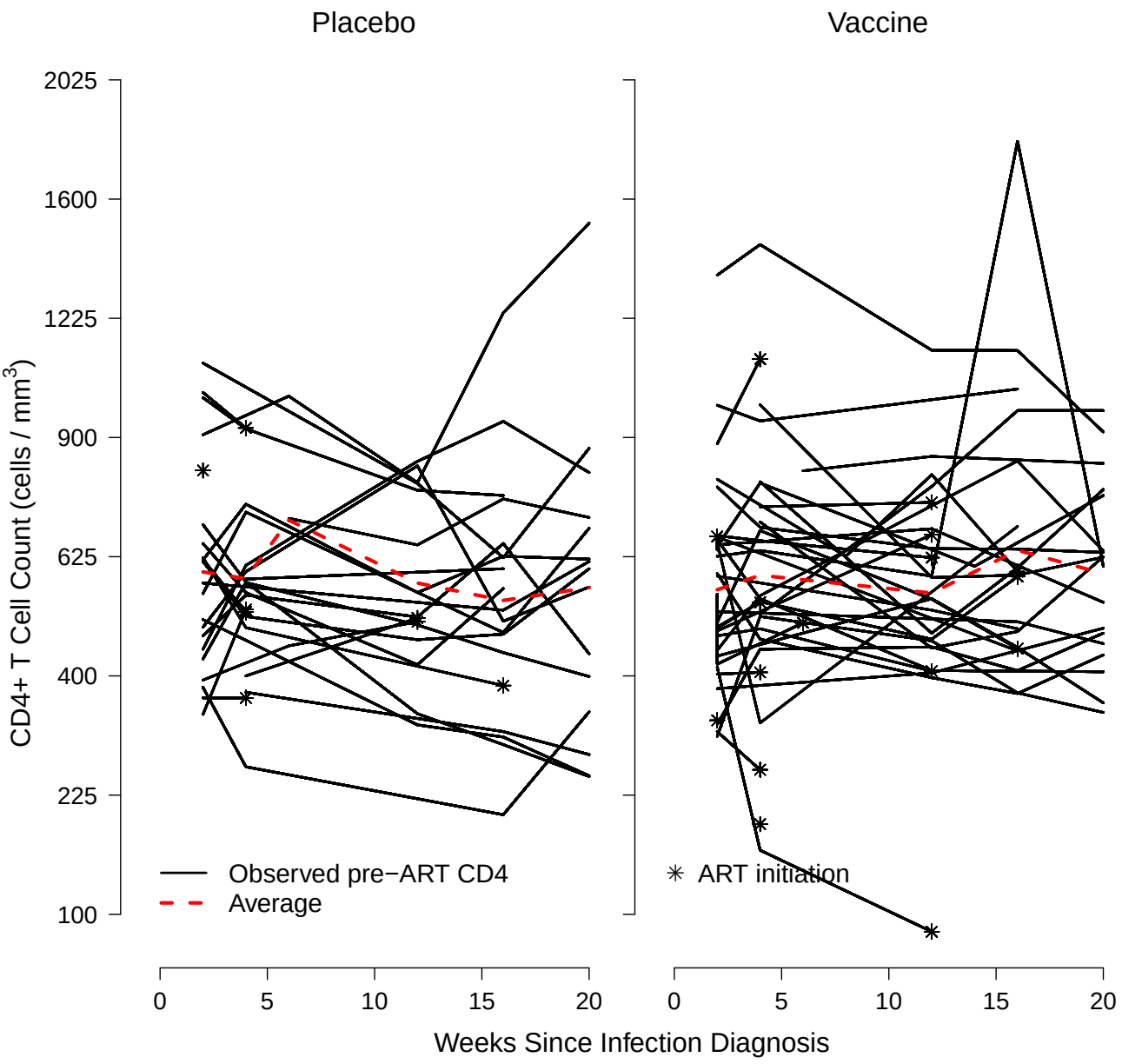


Figure S13: Longitudinal pre-ART CD4+ T cell count trajectories over weeks 2-20 post-infection diagnosis for individual MITT infected participants, by study arm. Red lines connect averages at each post-infection diagnosis visit with at least 3 observed values. Stars indicate participants whose viral load trajectories were censored by ART initiation.

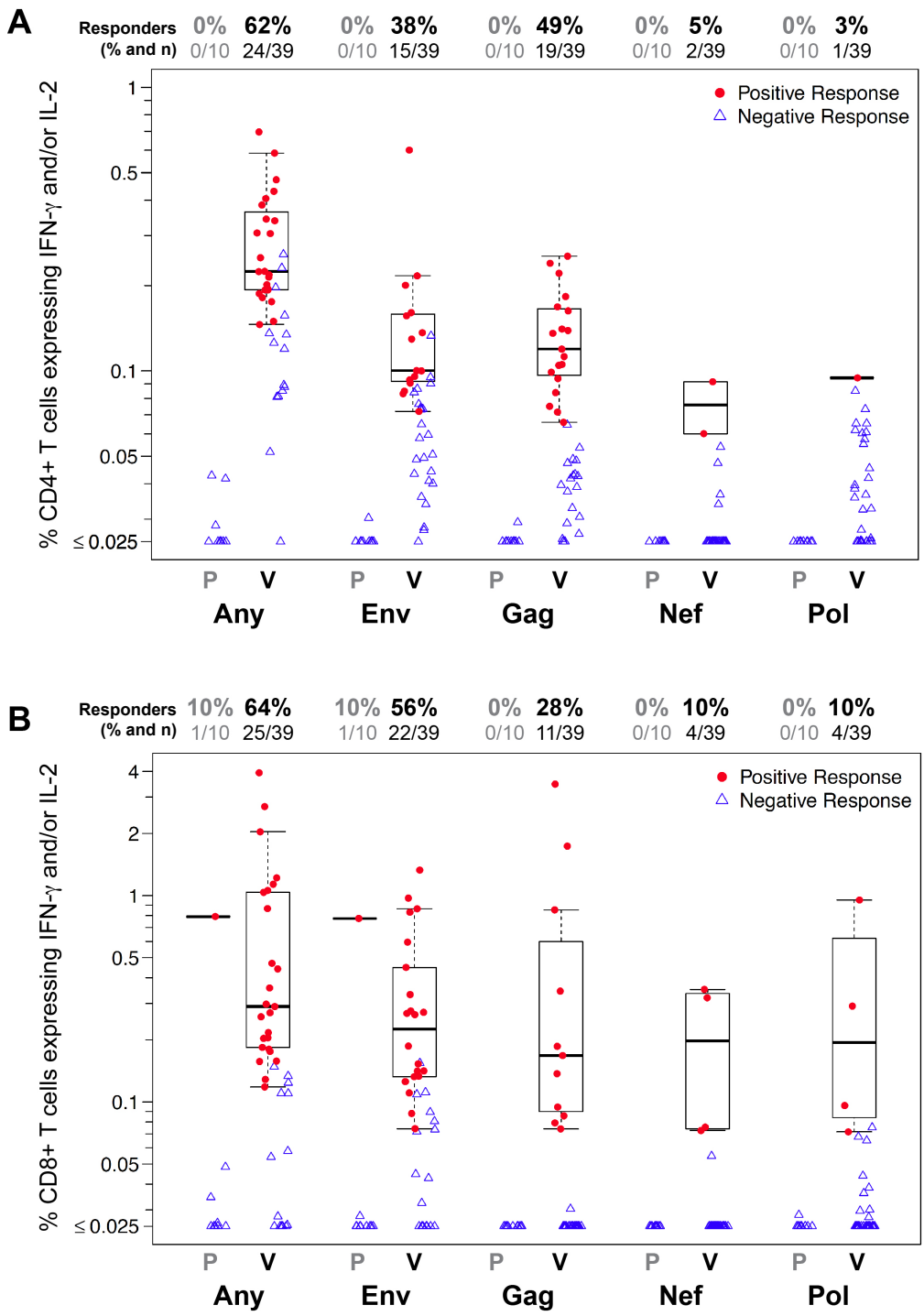


Figure S14: Ex vivo HIV-1-specific T-cell responses at visit 7 (Month 7) for vaccine recipients (V, n = 39) and placebo recipients (P, n = 10). Magnitude or response is measured by the net percentage of detectable, circulating (A) CD4+ and (B) CD8+ T cells that express IFN-γ and/or IL-2 intracellularly after stimulation with 15-mer peptide pools matched to the vaccine HIV-1 insert sequences. Positive responses are indicated by red circles, and negative by blue triangles. Percentages above the graphs represent response rates. The sum of responses to the individual proteins are shown on the far left above "Any". Boxplots show the distribution of net response among positive responders.

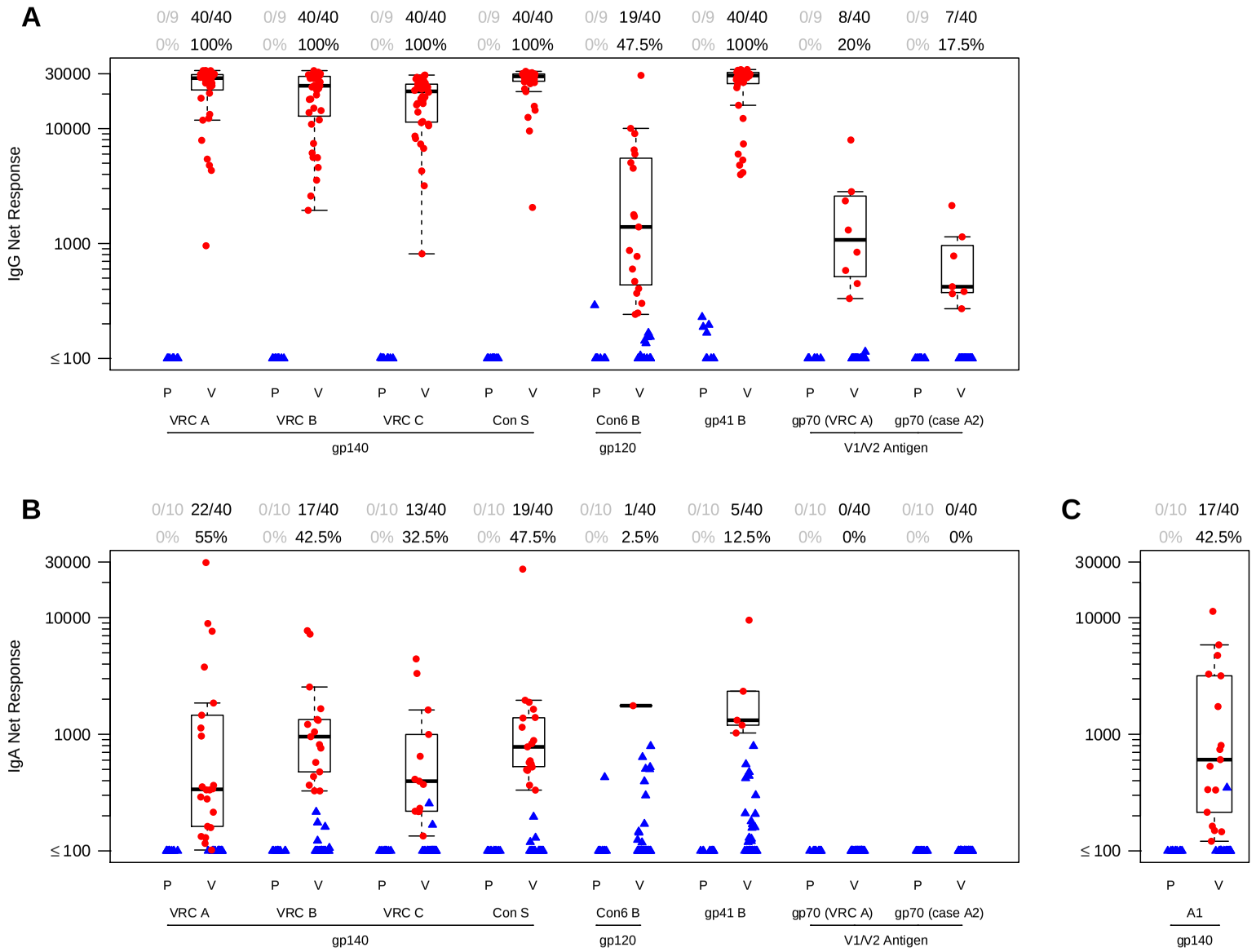


Figure S15: Binding antibody responses at visit 7 (Month 7) for vaccine recipients (V, n = 40) and placebo recipients (P, n = 10). Magnitudes of binding antibodies to Env antigens measured as mean fluorescence units (MFI) at 1:50 serum dilution, subtracting MFI for the"Blank" experiments with no Env antigens. (A) shows IgG net response to 8 Env antigens including the V1V2 antigen used in the RV144 [gp70 Case A2], and the V1V2 antigen contained in the VRC A vaccine strain; (B) shows IgA net response to the same 8 Env antigens; and (C) shows IgA net response to the A1.Congp140 Env used in RV144. Positive responses are indicated by red circles, and negative by blue triangles. Boxplots show the distribution of net response among positive responders.

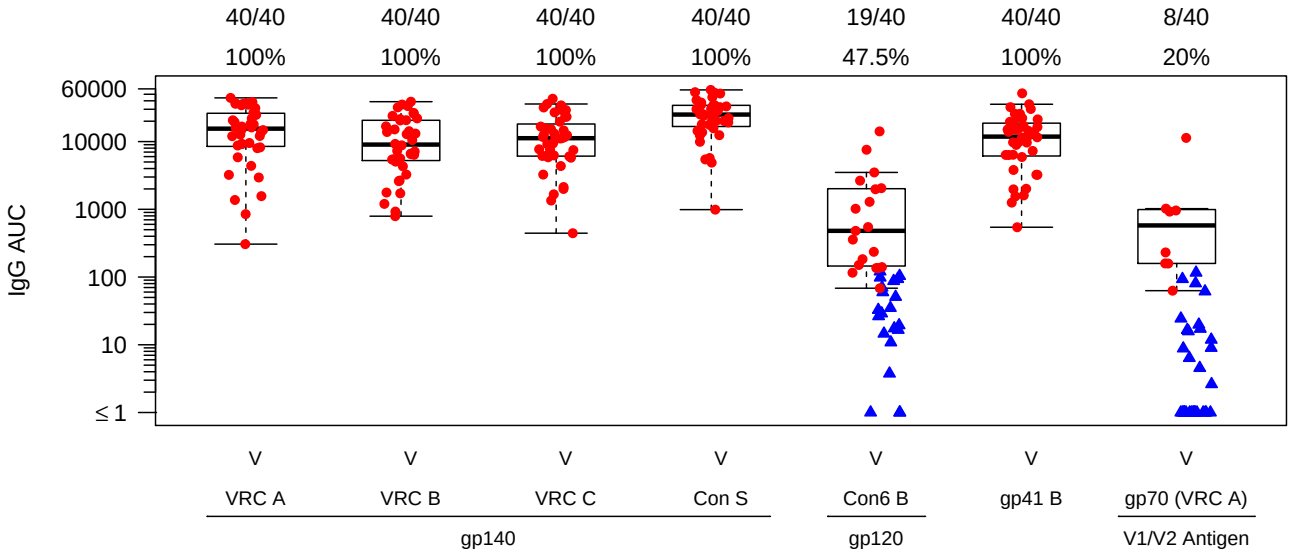


Figure S16: IgG binding antibody AUCs against Env at visit 7 (Month 7) for vaccine recipients to 7 of the 8 Env antigens assessed in Figure S15. Positive responses are indicated by red circles, and negative by blue triangles. Boxplots show the distribution of titers among positive responders.

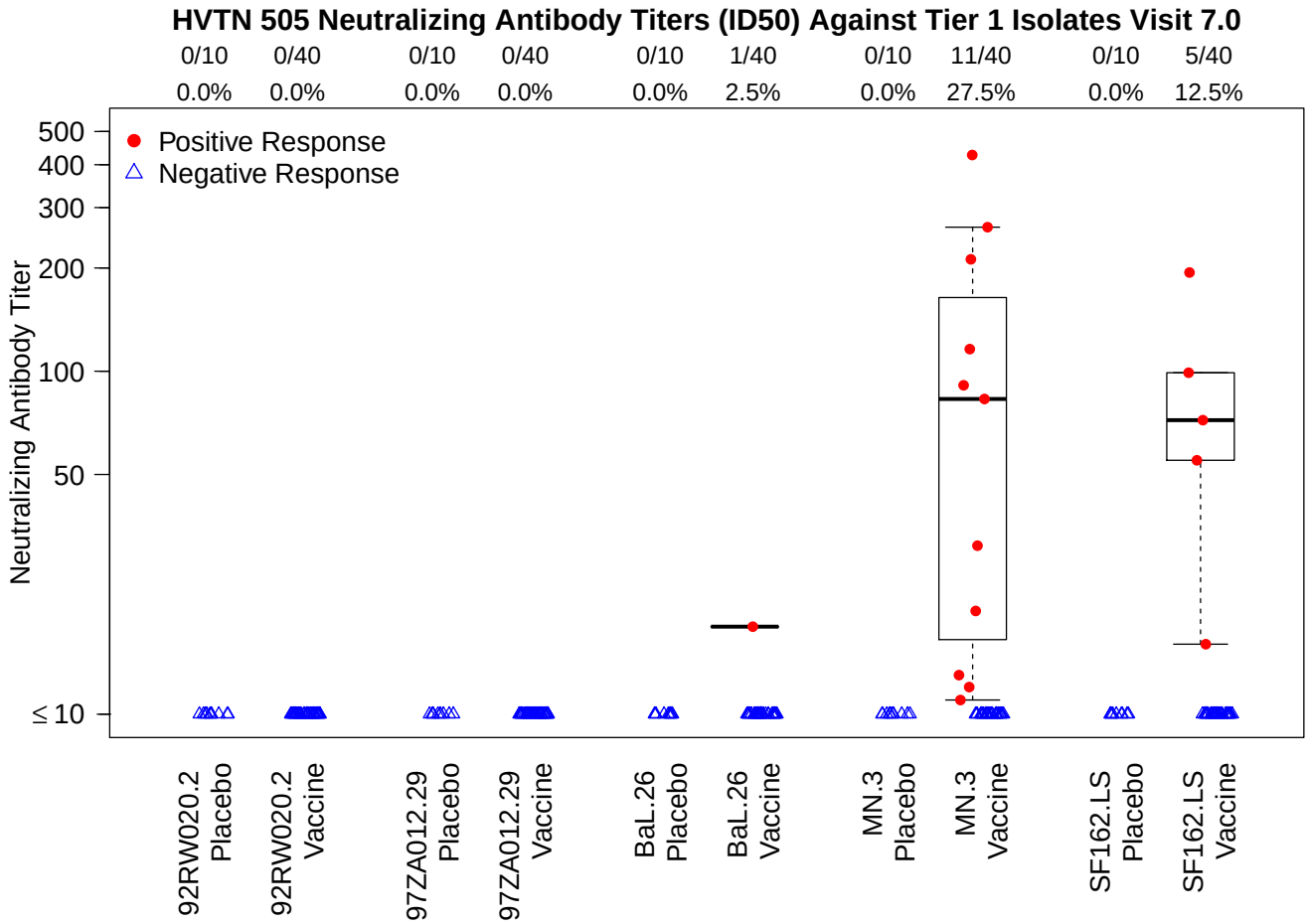
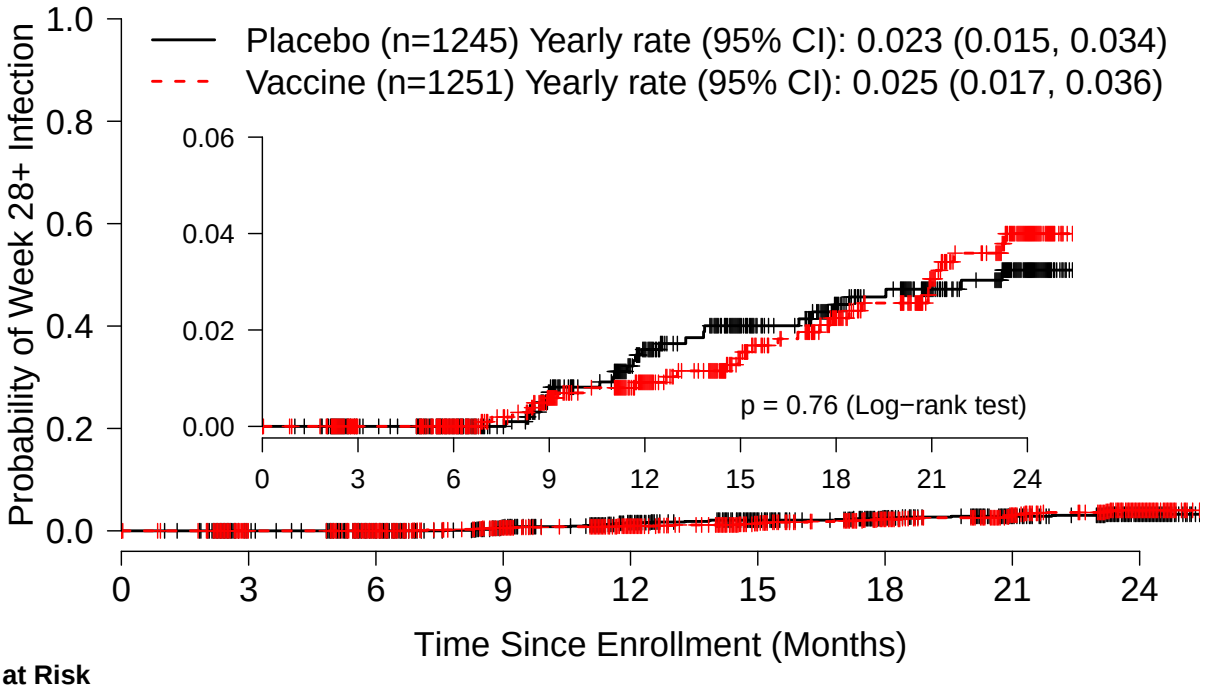
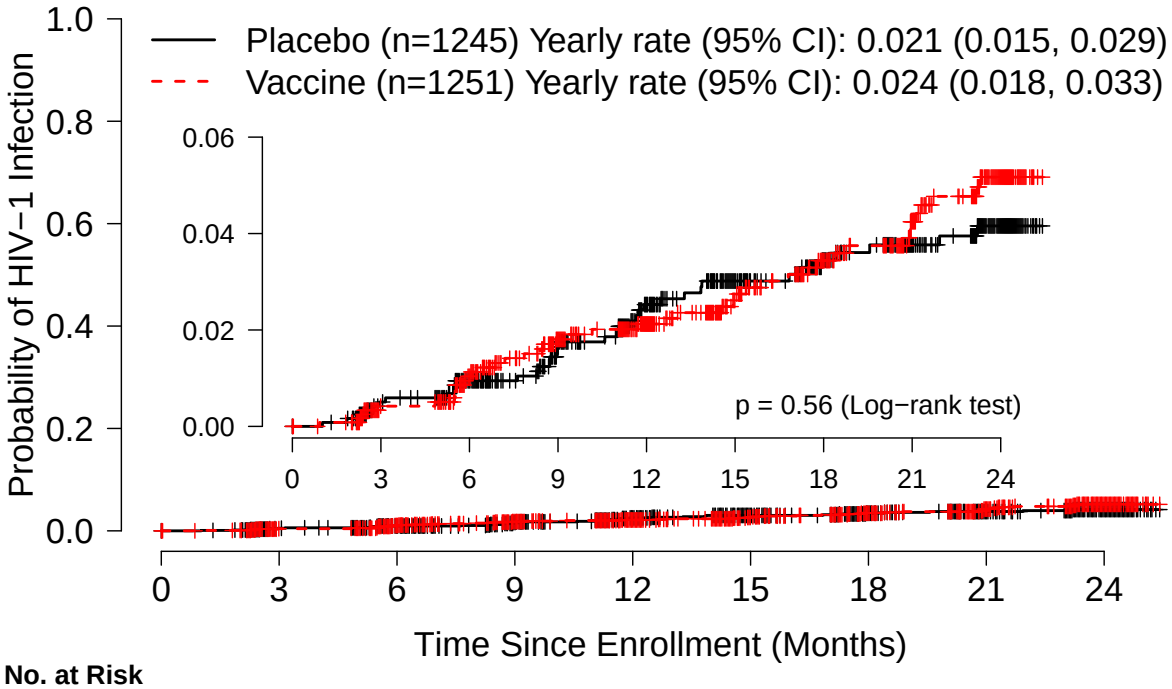


Figure S17: Neutralizing antibody titers against Tier 1 isolates at visit 7 (Month 7) by study arm. Positive responses are indicated by red circles, and negative by blue triangles. Boxplots show the distribution of titers among positive responders.



No. at Risk							
Placebo	1080	945	837	713	630	543	320
Vaccine	1088	961	856	737	643	563	342
Cumulative No. of Infections							
Placebo	0	7	15	19	22	24	26
Vaccine	0	6	9	13	19	24	29

Figure S18: Cumulative incidence of Week 28+ HIV-1 infection based on updated data through August 23, 2013.



No. at Risk

Placebo	1245	1152	1080	945	837	713	630	543	320
Vaccine	1251	1160	1088	961	856	737	643	563	342

Cumulative No. of Infections

Placebo	0	6	11	18	26	30	33	35	37
Vaccine	0	5	12	20	23	27	33	38	43

Figure S19: Cumulative incidence of MITT HIV-1 infection based on updated data through August 23, 2013.

Tables

Table S1: Baseline characteristics of MITT subjects.

	Vaccine ($n = 1251$) $n(\%)$	Placebo ($n = 1245$) $n(\%)$	All subjects ($n = 2496$) $n(\%)$
Gender Identity			
Male	1229 (98.2)	1223 (98.2)	2452 (98.2)
Transgender	22 (1.8)	22 (1.8)	44 (1.8)
Age			
Median (min, max)	29 (18, 50)	30 (18, 50)	29 (18, 50)
18 - 20 yr	106 (8.5)	108 (8.7)	214 (8.6)
21 - 30 yr	582 (46.5)	566 (45.5)	1148 (46.0)
31 - 40 yr	299 (23.9)	297 (23.9)	596 (23.9)
41 - 50 yr	264 (21.1)	274 (22.0)	538 (21.6)
Race/Ethnicity			
White	862 (68.9)	876 (70.4)	1738 (69.6)
Black/African American	202 (16.1)	195 (15.7)	397 (15.9)
Hispanic	115 (9.2)	85 (6.8)	200 (8.0)
Asian	12 (1.0)	20 (1.6)	32 (1.3)
Other	60 (4.8)	69 (5.5)	129 (5.2)
BMI [1]			
≤ 25.34	619 (49.5)	629 (50.5)	1248 (50.0)
> 25.34	631 (50.4)	615 (49.4)	1246 (49.9)
Missing	1 (0.1)	1 (0.1)	2 (0.1)
Behavioral Risk [2]			
Risk score [3]			
Low risk	454 (36.3)	465 (37.3)	919 (36.8)
Low-med. risk	337 (26.9)	313 (25.1)	650 (26.0)
Med.-high risk	208 (16.6)	209 (16.8)	417 (16.7)
High risk	252 (20.1)	258 (20.7)	510 (20.4)
Number male partners			
0	59 (4.7)	64 (5.1)	123 (4.9)
1	256 (20.5)	242 (19.4)	498 (20.0)
2	253 (20.2)	242 (19.4)	495 (19.8)
3 - 4	349 (27.9)	367 (29.5)	716 (28.7)
≥ 5	328 (26.2)	322 (25.9)	650 (26.0)
Missing	6 (0.5)	8 (0.6)	14 (0.6)
Had HIV+ male partner			

[1] BMI denotes body mass index.

[2] Behavioral risk over 3 months prior to enrollment was assessed using questionnaire.

[3] Risk score is a weighted average of two variables identified to predict HIV-1 infection risk using all subjects model selection: an indicator that the number of male sexual partners was greater than 3 and an indicator of unprotected receptive anal sex. Each variable is weighted by its estimated relative hazard ratio for HIV-1 infection. The risk score is 0 if neigher risk factor is reported, 1 if both are reported, and intermediate (0.54 or 0.46) if one of the two is reported.

	Vaccine ($n = 1251$) $n(\%)$	Placebo ($n = 1245$) $n(\%)$	All subjects ($n = 2496$) $n(\%)$
Yes	233 (18.6)	212 (17.0)	445 (17.8)
Missing	6 (0.5)	8 (0.6)	14 (0.6)
Unprotected insertive anal sex			
Yes	673 (53.8)	693 (55.7)	1366 (54.7)
Missing	15 (1.2)	10 (0.8)	25 (1.0)
Unprotected receptive anal sex			
Yes	587 (46.9)	568 (45.6)	1155 (46.3)
Missing	14 (1.1)	13 (1.0)	27 (1.1)
Female partner			
Yes	117 (9.4)	129 (10.4)	246 (9.9)
Missing	16 (1.3)	15 (1.2)	31 (1.2)
Trans. partner [4]			
Yes	40 (3.2)	42 (3.4)	82 (3.3)
Missing	17 (1.4)	16 (1.3)	33 (1.3)
Sex for money/gifts			
Yes	49 (3.9)	59 (4.7)	108 (4.3)
Missing	7 (0.6)	8 (0.6)	15 (0.6)
Gonorrhea			
Yes	55 (4.4)	49 (3.9)	104 (4.2)
Unknown	31 (2.5)	22 (1.8)	53 (2.1)
Missing	19 (1.5)	16 (1.3)	35 (1.4)
Genital sores/discharge			
Yes	50 (4.0)	35 (2.8)	85 (3.4)
Missing	21 (1.7)	16 (1.3)	37 (1.5)
Recreational drug use			
Marijuana	535 (42.8)	557 (44.7)	1092 (43.8)
Other [5]	524 (41.9)	538 (43.2)	1062 (42.5)
Missing	192 (15.3)	150 (12.0)	342 (13.7)
Alcohol use			
Never	131 (10.5)	142 (11.4)	273 (10.9)
$<$ 1/wk	328 (26.2)	344 (27.6)	672 (26.9)
1 - 2 days/wk	434 (34.7)	410 (32.9)	844 (33.8)
3 - 6 days/wk	296 (23.7)	281 (22.6)	577 (23.1)
Daily	45 (3.6)	55 (4.4)	100 (4.0)
Missing	17 (1.4)	13 (1.0)	30 (1.2)

[4]Trans. denotes transgender (self-identified).

[5]Other recreational drugs include: poppers, crack cocaine, powdered cocaine, speed, erectile dysfunction drugs, ecstasy, and all other recreational drugs.

Table S2: Number (%) of enrolled participants experiencing local reactogenicities, systemic reactogenicities, and adverse events by study arm. Two-sided Fisher's exact tests were used to compare rates of any local reactogenicity, any systemic reactogenicity, any adverse event, and any of the above rated severe or greater between study arms.

		Vaccine ($n = 1253$)	Placebo ($n = 1251$)	p-value
Max. local reactogenicity				
	Any	1132 (90.3%)	881 (70.4%)	< 0.001
	Any severe or greater	22 (1.8%)	2 (0.2%)	< 0.001
	None	121 (9.7%)	370 (29.6%)	
	Mild	734 (58.6%)	789 (63.1%)	
	Moderate	376 (30.0%)	90 (7.2%)	
	Severe	22 (1.8%)	2 (0.2%)	
	Life threatening	0 (0%)	0 (0%)	
Max. systemic reactogenicity				
	Any	723 (57.7%)	605 (48.4%)	< 0.001
	Any severe or greater	45 (3.6%)	12 (1.0%)	< 0.001
	None	530 (42.3%)	646 (51.6%)	
	Mild	432 (34.5%)	419 (33.5%)	
	Moderate	246 (19.6%)	174 (13.9%)	
	Severe	44 (3.5%)	12 (1.0%)	
	Life threatening	1 (0.1%)	0 (0%)	
Adverse events				
	Any	718 (57.3%)	686 (54.8%)	0.23
	Any severe or greater	40 (3.2%)	41 (3.3%)	0.91
	None	535 (42.7%)	565 (45.2%)	
	Mild	415 (33.1%)	382 (30.5%)	
	Moderate	263 (21.0%)	263 (21.0%)	
	Severe	35 (2.8%)	36 (2.9%)	
	Life threatening	5 (0.4%)	4 (0.3%)	
	Fatal	0 (0%)	1 (0.1%)	

Table S3: Number and percent of participants experiencing different types of adverse events by study arm. Adverse events are classified according to MedDRA system organ classes and preferred terms. Adverse events for which unadjusted $p < 0.05$ from two-sided Fisher's exact tests comparing study arms are shown. The adjusted p-values, calculated using the DFDR method of (Mehrotra and Adewale, 2012), are also shown. Adverse events with adjusted p-values less than 0.20 are "flagged" by this procedure.

System Organ Class	Preferred Term	n Vaccine	% Vaccine	n Placebo	% Placebo	Unadj. p	Adj. p	Flagged
General disorders and administration site conditions	Influenza like illness	1	0.10	6	0.50	0.07	1.00	
General disorders and administration site conditions	Injection site pruritus	19	1.50	1	0.10	< 0.001	0.002	***
Infections and infestations	Acarodermatitis	1	0.10	7	0.60	0.04		
Injury, poisoning and procedural complications	Contusion	10	0.80	3	0.20	0.09		
Injury, poisoning and procedural complications	Ligament sprain	7	0.60	1	0.10	0.07		
Musculoskeletal and connective tissue disorders	Muscle spasms	5	0.40	0	0.00	0.06		
Nervous system disorders	Dizziness	20	1.60	9	0.70	0.06		
Reproductive system and breast disorders	Erectile dysfunction	0	0.00	4	0.30	0.06		
Respiratory, thoracic and mediastinal disorders	Oropharyngeal pain	37	3.00	23	1.80	0.09		
Skin and subcutaneous tissue disorders	Rash erythematous	0	0.00	4	0.30	0.06		

Table S4: Estimated Week 28+ HIV-1 incidence and vaccine efficacy for preventing Week 28+ HIV-1 infection using partial likelihood estimation, by baseline subject characteristics.

Variable	Placebo				Vaccine				Vaccine Efficacy
	n	No. with Inf.	No. Person-yrs	Rate[a]	n	No. with Inf.	No. Person-yrs	Rate[a]	% (95% CI)
Overall	1245	21	1508.5	0.014	1251	27	1539.3	0.018	-25.04 (-121.17, 29.30)
Risk score (quantitative)[b]									
Low risk (score = 0)									11.41 (-166.34, 70.53)
Low-med risk (score = 0.46)									-14.51 (-112.11, 38.18)
Med-high risk (score = 0.54)									-19.74 (-114.36, 33.12)
High risk (score = 1)									-54.77 (-257.52, 33.00)
Risk score (categorical)[c]									
Low risk (score = 0)	465	3	574.3	0.005	454	5	566.8	0.009	-69.16 (-607.85, 59.57)
Low-med risk (score = 0.46)	313	6	371.2	0.016	337	5	397.6	0.013	22.99 (-152.35, 76.50)
Med-high risk (score = 0.54)	209	6	254.4	0.024	208	4	267.9	0.015	37.74 (-120.64, 82.43)
High risk (score = 1)	258	6	308.6	0.019	252	13	307.1	0.042	-114.36 (-463.99, 18.53)
Race/Ethnicity									
Non-White and/or Hispanic	369	7	472.6	0.015	389	12	480.3	0.025	-70.91 (-334.10, 32.71)
Non-Hispanic White	876	14	1035.9	0.014	862	15	1059.1	0.014	-3.02 (-113.43, 50.27)
Age (years)									
Age $\leq$ 29	621	12	724.4	0.017	646	15	760.8	0.020	-19.87 (-156.10, 43.90)
Age $>$ 29	624	9	784.1	0.011	605	12	778.5	0.015	-31.36 (-211.77, 44.65)
Body Mass Index (BMI)									
BMI $\leq$ 25.34	629	16	741.0	0.022	619	15	750.1	0.020	8.71 (-84.66, 54.87)
BMI $>$ 25.34	615	5	765.6	0.007	631	12	787.2	0.015	-133.19 (-561.91, 17.85)

[a]Rate = No. with Infection / No. Person-yrs

[b] For quantitative risk score analysis, the results in each row show model-based VE estimates for subjects with risk score equal to the indicated value.

[c] For categorical risk score analysis, the results in each row show incidence/VE among subjects with risk score equal to the indicated value.

Table S5: Estimated MITT HIV-1 incidence and vaccine efficacy for preventing MITT HIV-1 infection using partial likelihood estimation, by baseline subject characteristics.

Variable	Placebo				Vaccine				Vaccine Efficacy
	n	No. with Inf.	No. Person-yrs	Rate[a]	n	No. with Inf.	No. Person-yrs	Rate[a]	% (95% CI)
Overall	1245	31	1508.5	0.021	1251	41	1539.3	0.027	-29.38 (-106.28, 18.85)
Risk score (quantitative)[b]									
Low risk (score = 0)									13.31 (-128.22, 67.07)
Low-med risk (score = 0.46)									-14.93 (-95.34, 32.38)
Med-high risk (score = 0.54)									-20.71 (-97.13, 26.09)
High risk (score = 1)									-60.03 (-207.72, 16.78)
Risk score (categorical)[c]									
Low risk (score = 0)	465	5	574.3	0.009	454	6	566.8	0.011	-21.45 (-297.93, 62.94)
Low-med risk (score = 0.46)	313	6	371.2	0.016	337	9	397.6	0.023	-39.69 (-292.46, 50.28)
Med-high risk (score = 0.54)	209	8	254.4	0.031	208	4	267.9	0.015	52.71 (-57.06, 85.76)
High risk (score = 1)	258	12	308.6	0.039	252	22	307.1	0.072	-84.15 (-272.10, 8.87)
Race/Ethnicity									
Non-White and/or Hispanic	369	9	472.6	0.019	389	16	480.3	0.033	-74.82 (-295.61, 22.75)
Non-Hispanic White	876	22	1035.9	0.021	862	25	1059.1	0.024	-10.90 (-96.68, 37.47)
Age (years)									
Age $\leq$ 29	621	16	724.4	0.022	646	23	760.8	0.030	-36.92 (-159.17, 27.67)
Age $>$ 29	624	15	784.1	0.019	605	18	778.5	0.023	-20.37 (-138.86, 39.34)
Body Mass Index (BMI)									
BMI $\leq$ 25.34	629	25	741.0	0.034	619	24	750.1	0.032	5.61 (-65.27, 46.09)
BMI $>$ 25.34	615	6	765.6	0.008	631	17	787.2	0.022	-175.84 (-599.63, -8.76)

[a]Rate = No. with Infection / No. Person-yrs

[b] For quantitative risk score analysis, the results in each row show model-based VE estimates for subjects with risk score equal to the indicated value.

[c] For categorical risk score analysis, the results in each row show incidence/VE among subjects with risk score equal to the indicated value.

Table S6: Reasons for missing setpoint viral load among Week 28+ and MITT infected participants evaluable for the setpoint viral load analysis. PD = post-infection diagnosis.

			Reason for missing setpoint viral load		
		Missing setpoint viral load n (%)	Dropped out prior to Week 10 PD n (%)	Initiated ART prior to Week 10 PD n (%)	Missed Weeks 10-20 PD visits n (%)
Week 28+	Placebo (n = 20)	3 (15)	1 (5)	2 (10)	0 (0)
	Vaccine (n = 27)	5 (19)	1 (4)	4 (15)	0 (0)
	Total (n = 47)	8 (17)	2 (4)	6 (13)	0 (0)
MITT	Placebo (n = 29)	6 (21)	1 (3)	4 (14)	1 (3)
	Vaccine (n = 40)	6 (15)	2 (5)	4 (10)	0 (0)
	Total (n = 69)	12 (17)	3 (4)	8 (12)	1 (1)

Table S7: Estimated difference in mean setpoint viral load (Δ VL) between placebo and vaccine Week 28+ infected participants by baseline covariates.

Variable	Placebo Mean VL (95% CI)	Vaccine Mean VL (95% CI)	Δ VL (95% CI)
Overall	4.47 (4.07, 4.94)	4.46 (3.97, 4.86)	0.00 (-0.55, 0.68)
Risk score (categorical)			
Low risk (score = 0)	4.46 (4.23, 4.69)	4.96 (4.42, 5.70)	-0.50 (-1.31, 0.14)
Low-med risk (score = 0.46)	4.29 (3.44, 5.37)	4.58 (3.93, 5.50)	-0.29 (-1.30, 0.92)
Med-high risk (score = 0.54)	4.68 (4.01, 5.49)	2.87 (1.93, 5.10)	1.80 (-0.67, 3.04)
High risk (score = 1)	4.59 (3.19, 5.81)	4.41 (3.75, 4.98)	0.18 (-1.18, 1.39)
Race/Ethnicity			
Non-White and/or Hispanic	4.52 (3.98, 5.06)	4.06 (3.25, 4.48)	0.45 (-0.21, 1.42)
Non-Hispanic White	4.43 (3.81, 5.19)	4.90 (4.22, 5.30)	-0.47 (-1.10, 0.62)
Age (years)			
Age $\leq$ 29	4.32 (3.79, 4.80)	3.99 (3.40, 4.59)	0.33 (-0.46, 1.05)
Age $>$ 29	4.70 (3.98, 5.62)	4.98 (4.31, 5.34)	-0.28 (-0.99, 0.88)
Body Mass Index (BMI)			
BMI $\leq$ 25.34	4.37 (3.91, 4.75)	4.59 (3.85, 5.21)	-0.22 (-0.98, 0.58)
BMI $>$ 25.34	5.62 (4.89, 6.35)	4.20 (3.66, 4.66)	1.42 (0.46, 2.58)

Table S8: Estimated difference in mean setpoint viral load (Δ VL) between placebo and vaccine MITT infected participants by baseline covariates.

Variable	Placebo Mean VL (95% CI)	Vaccine Mean VL (95% CI)	Δ VL (95% CI)
Overall	4.54 (4.22, 4.85)	4.51 (4.17, 4.81)	0.03 (-0.42, 0.48)
Risk score (categorical)			
Low risk (score = 0)	4.52 (4.23, 4.69)	4.76 (4.12, 5.40)	-0.24 (-0.96, 0.43)
Low-med risk (score = 0.46)	4.29 (3.44, 5.37)	4.31 (3.79, 4.96)	-0.03 (-0.97, 1.13)
Med-high risk (score = 0.54)	4.77 (3.94, 5.59)	2.87 (1.93, 5.10)	1.89 (-0.60, 3.16)
High risk (score = 1)	4.49 (3.97, 4.98)	4.56 (4.13, 4.94)	-0.07 (-0.71, 0.60)
Race/Ethnicity			
Non-White and/or Hispanic	4.55 (4.07, 5.01)	4.20 (3.48, 4.63)	0.35 (-0.22, 1.21)
Non-Hispanic White	4.51 (4.07, 4.91)	4.69 (4.28, 5.10)	-0.19 (-0.76, 0.40)
Age (years)			
Age $\leq$ 29	4.38 (3.95, 4.76)	4.36 (3.74, 4.84)	0.02 (-0.64, 0.75)
Age $>$ 29	4.64 (4.17, 5.26)	4.64 (4.20, 5.03)	0.01 (-0.56, 0.79)
Body Mass Index (BMI)			
BMI $\leq$ 25.34	4.46 (4.09, 4.75)	4.52 (3.93, 4.90)	-0.05 (-0.60, 0.57)
BMI $>$ 25.34	5.32 (4.62, 6.35)	4.47 (3.96, 4.92)	0.85 (-0.05, 2.03)

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